syngenta

Event SYHT0H2 Soybean

The Effect of Diets Containing Soybean Meal from SYHT0H2, Nontransgenic Control and a Commercial Variety on Broiler Growth Performance and Carcass Parameters

Final Report

DATA REQUIREMENT(S): Not Applicable

AUTHOR(S): [redacted]

STUDY COMPLETION DATE: July 24, 2012

PERFORMING LABORATORY:
Colorado Quality Research, Inc.
400 E. County Road 72
Wellington, CO 80549
(970) 568-7738 phone

LABORATORY PROJECT ID:
Report Number: SYN-11-1
Syngenta Study No. TK0036575

SUBMITTER:
Syngenta Seeds, Inc.
3054 East Cornwallis Road
Post Office Box 12257
Research Triangle Park, NC 27709-2257 USA

SPONSOR:
Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

The following statement applies to submissions to the United States Environmental Protection Agency (US EPA).

No Claim of Confidentiality

No claim of confidentiality is made for any information contained in this report on the basis of its falling within the scope of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 10 (d) (1) (A), (B), or (C).

Company: *Syngenta Seeds, Inc.*

Company Representative:

[redacted]

24 July 2012

Date

Manager, Regulatory Affairs

These data are the property of Syngenta Seeds, Inc. and, as such, are considered to be confidential for all purposes other than compliance with the regulations implementing FIFRA Section 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other provision of common law or statute or in any other country.

The following statement applies to submissions to regulatory agencies and other competent authorities other than the US EPA and all other viewers.

This Document Contains Confidential Business Information

This document contains information that is proprietary to Syngenta and, as such, is considered to be confidential for all purposes other than compliance with the relevant registration procedures.

Without the prior written consent of Syngenta, this information may (i) not be used by any third party including, but not limited to, any other regulatory authority for the support of regulatory approval of this product or any other product, and (ii) not be published or disclosed to any third party including, but not limited to, any authority for the support of regulatory approval of any products.

Its submission does not constitute a waiver of any right to confidentiality that may exist in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the relevant provisions of Good Laboratory Practice Standards (40 CFR Part 160, US EPA 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act, and subsequent revisions. However, the study was conducted according to accepted scientific methods, and the raw data and study records have been retained.

The in-life portion of the study meets the Good Laboratory Practice (GLP) requirements for 21 CFR Part 58. Portions of the study conducted by Syngenta meet the GLP requirements for 40 CFR Part 160. Specific items that were not conducted under GLP include:

- Diet analyses at the University of Missouri Experiment Station Chemical Laboratories
- Nutritional Component Analysis of soybean meal at Covance Laboratories, Inc.
- Water analyses
- Total coliform analysis of test facility water by Stewart Environmental Consultants, Inc.
- Northern Colorado Water Association water testing
- Dr Bob Buresh diet formulations
- Yearly scale licensing by the State of Colorado
- Stability of the test, control, and commercial materials and the stability, uniformity and concentration of the test, control, and commercial materials, as a component in diets

These exceptions had no effect on the integrity or quality of the study.

Colorado Quality Research subcontracted to Integrated Quality Management (Ms. Catherine Bens) to conduct inspections, data and report audits as necessary to ensure the integrity of the data generated by CQR. Syngenta QAU conducted data and report audits as necessary to ensure the integrity of the analytical data generated by Syngenta. Written reports of inspections/audit findings were reported to the Study Director and Testing Facility Management (at a minimum).

Study Director:

[redacted]

24JUL12
Date

Colorado Quality Research, Inc.,
400 E. County Road 72, Wellington, CO 80549 USA

Submitted by:

[redacted]

24 July 2012
Date

Syngenta Seeds, Inc.
3054 East Cornwallis Road, Post Office Box 12257, Research Triangle Park, NC 27709-2257 USA

Sponsor:

[redacted]

24 July 2012
Date

Syngenta Crop Protection, LLC
410 Swing Road, Post Office Box 18300, Greensboro, NC 27419-8300 USA

QUALITY ASSURANCE STATEMENT

Study Title: The effect of diets containing soybean meal from SYHT0H2, nontransgenic control and a commercial variety on broiler growth performance and carcass parameters

Study Number: SYN-11-1/Syngenta TK0036575

Reviews conducted by the Quality Assurance Unit confirm that the final report accurately describes the methods and standard operating procedures followed and accurately reflects the raw data for the portion of the study conducted by Colorado Quality Research, Inc. (CQR).

Following is a list of reviews conducted by Integrated Quality Management on the study reported herein. The original Quality Assurance Reports will be retained with the Monsanto QAU Record.

Dates of Inspection / Audit	Phase	Date Reported To:	
		Study Director	Management
01/18/2012	Protocol Review	01/19//2012	01/19/2012
01/26/2012	Treatment Diets - Preparation & Sampling	02/15/2012	02/15/2012
02/01/2012	Start Study (Day 0)	02/15/2012	02/15/2012
02/22/2012	Feed change (Day 21)	02/22/2012	02/22/2012
03/14/2012	Pre-Processing Feed and Body Weight (Day 42)	03/14/2012	03/14/2012
03/15/2012	Processing (Day 43)	03/16/2012	03/16/2012
03/21-26/2012	Raw Data Inspection	03/29/2012	03/29/2012
03/28-29/2012	Statistical Analysis Subreport Inspection	03/29/2012	03/29/2012
05/23-25/2012	Statistical Analysis Subreport Inspection	05/30/2012	05/30/2012
06/07-12/2012	Draft and Final Report Inspection	06/12/2012	06/12/2012

Following is a list of reviews conducted by Syngenta Crop Protection, LLC on the Analytical Phase Report of the study.

Dates of Inspection / Audit	Phase	Date Reported To:	
		Study Director	Management
03/01/2012	Inspect Analytical	03/02/2012	03/02/2012
04/19-23/2012	Audit Phase Report (1st Audit)	04/24/2012	04/24/2012
05/22/2012	Audit Phase Report (2nd Audit)	05/23/2012	05/23/2012

[redacted]

24 July 2012
Date

Contract Quality Assurance
Integrated Quality Management

STUDY DIRECTOR'S COMMENTS/CERTIFICATION STATEMENT

No adverse effects were observed. There were no known circumstances that may have affected the data quality or integrity.

I, [redacted], Study Director, attest that Study No. SYN-11-1 (Syngenta No. TK0036575) was conducted according to the Protocol, amendments and deviations and that the data were collected and recorded in accordance with the applicable Food and Drug Administration, Center for Veterinary Medicine (CVM) Guidelines.

[redacted]

Study Director

24 JUL 12

Date

TABLE OF CONTENTS

LIST OF TABLES

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
[redacted]	Study Director, Colorado Quality Research, Inc.
[redacted]	Study Monitor, Syngenta Crop Protection, LLC
[redacted]	Principal Investigator, Syngenta Crop Protection, LLC
[redacted]	Sponsor, Syngenta Crop Protection, LLC
[redacted]	Test Facility Management, Colorado Quality Research, Inc.

Study dates

Study Initiation (Protocol signed): January 19, 2012
Study Completion (Report signed): July 24, 2012

Records Retention

Originals of study specific raw data generated at Colorado Quality Research, Inc., and the Statistician's report are retained at Syngenta, 3054 East Cornwallis Road, Durham, NC 27709-2257 USA. Original records from the University of Missouri Experiment Station Chemical Laboratories (ESCL) and Covance Laboratories Inc. are retained at ESCL, Columbia, MO, and Covance, Madison, WI 53704 respectively.

1.0 EXECUTIVE SUMMARY

A 42-day broiler feeding study evaluated whether standard poultry diets prepared with soybean meal from Event SYHT0H2 transgenic soybean had an effect on male and female broiler chicken survival, feed consumption, growth, feed conversion, or carcass characteristics as compared to diets prepared with a soybean meal from a nontransgenic, near-isogenic control soybean variety, or diets prepared with soybean meal from a commercial nontransgenic reference soybean variety. SYHT0H2 transgenic soybean plants contain the genes encoding the *p*-hydroxyphenylpyruvate dioxygenase AvHPPD-03 protein and the phosphinothrin acetyltransferase (PAT) protein.

Starter and grower/finisher diets were prepared using toasted soybean meal from each source and were formulated to meet the nutritional requirements of broilers for each stage of growth. The soybean meal content of the diets ranging from approximately 29% to 33.5% by weight. The soybean meal processed from SYHT0H2 soybean was expected to be nutritionally equivalent to conventional soybean meal.

This study was conducted to evaluate the nutritional value of diets containing a replacement amount of soybean meal produced from SYHT0H2 soybean. The performance of broilers fed SYHT0H2 soybean containing diets for 42 days was compared to the performance of broilers fed diets containing soybean meal processed from a nontransgenic control or the commercial soybean variety.

Performance over the 42-day test period of broilers fed diets containing SYHT0H2 soybean meal was not different ($P > 0.05$) than that of broilers fed diets formulated with nontransgenic control soybean meal or commercial soybean meal. No treatment-by-sex interactions were detected ($P > 0.05$) for performance parameters across the test and control or commercial soybean meal-fed birds. Measures of broiler performance were of similar magnitude for broilers fed diets formulated to the same nutrient specifications using the soybean meal component of the diet provided by SYHT0H2, nontransgenic control, or commercial soybean meal. No unexpected effects on broilers were observed when broilers were fed diets containing soybean meal produced from SYHT0H2 soybean compared to diets containing nontransgenic control or commercial soybean meal. Mortality was low for all three treatment groups and for SYHT0H2 and the nontransgenic control meal-fed birds the mortality was equal.

There were no biologically relevant differences in broiler performance or carcass yield between broilers fed diets containing soybean meal produced from SYHT0H2 soybean and those fed diets containing nontransgenic control soybean meal or conventional commercial material.

The diets containing soybean meal produced from SYHT0H2 soybean were as wholesome as the diets containing nontransgenic control or commercial soybean meal regarding their ability to support the rapid growth of broiler chickens. These data support the conclusion that soybean meal produced from SYHT0H2 soybean is as nutritious as nontransgenic soybean meal.

2.0 INTRODUCTION

Soybean (*Glycine max* [L.] Merrill) has been genetically modified to express the novel genes *avhppd-03* derived from oat (*Avena sativa* L.) and *pat* derived from *Streptomyces viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03, that catalyzes the formation of homogentisic acid, the aromatic precursor in plastoquinone and vitamin E biosynthesis. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits endogenous HPPD. Expression of *avhppd-03* in transgenic Event SYHT0H2 soybean plants confers a mesotrione-tolerance phenotype. The gene *pat* encodes the enzyme phosphinothricin acetyltransferase (PAT), which inactivates the herbicide glufosinate-ammonium, an inhibitor of glutamine synthetase, an enzyme in the nitrogen assimilation pathway. Expression of *pat* confers a glufosinate-tolerance phenotype, which was used as a selectable marker in the development of SYHT0H2 soybean.

Toasted soybean meal derived from typical commercial soybean processing is used primarily for animal feed; approximately 98% is used as animal feed in the United States (Soyatech, 2012). The soybean meal processed from SYHT0H2 soybean was expected to be nutritionally equivalent to conventional soybean meal.

The purpose of this study was to evaluate whether broiler chickens fed diets prepared with SYHT0H2 transgenic soybean meal exhibited any adverse effects on survival, feed consumption, growth, feed conversion, or carcass characteristics when directly compared with broiler chickens fed diets prepared with nontransgenic, near-isogenic soybean meal. Diets prepared with soybean meal from nontransgenic, commercially available soybeans were used as an additional reference for comparison. Effects on any of the performance parameters could be due to the presence of the transgenic proteins in the diet or as a result of any unintended compositional changes in the meal that may have altered its nutritional value.

3.0 MATERIALS AND METHODS

3.1 Testing/Support Facilities

Facility / Contact	*Purpose*
Colorado Quality Research, Inc. 400 East County Road 72 Wellington, CO 80549	Test, control, and commercial material storage, feed preparation, archives (copies), test animal housing, In-life phase study conduct, including bird processing
Syngenta Crop Protection, LLC	Supplier of soybean meal, and archives (originals). Characterization of test, control, and commercial control articles (Under separate protocol through Food Protein Research and Development Center, Texas A&M University, College Station, TX)
[redacted] 890 Bren Del Dr Petoskey, MI 49770 [redacted]	Statistical analyses
[redacted] Integrated Quality Management 389 Big Sky Place Wellington, CO 80549- [redacted]	Quality Assurance Unit for portions conducted at CQR
[redacted] Consulting Nutritionist P.O. Box 1705 Clemson, SC 29633	Consulting nutritionist, diet formulation
Covance Laboratories, Inc. 3301 Kinsman Boulevard Madison, WI 53704	Nutritional Component Analysis of soybean meal
University of Missouri Attention: [redacted] Experiment Station Chemical Laboratories Room 4, Agriculture Building University of Missouri Columbia, MO 65211-7170	Ingredient and Diet analysis (for dietary nutrients)
Syngenta Biotechnology, LLC 3054 E Cornwallis Dr Durham, NC 27709, USA	Diet analysis for transgenic protein and DNA content

3.2 Test, Control and Reference Soybean Meal

Test Article: Transgenic SYHT0H2 soybean meal

Control Article: Nontransgenic, near-isogenic "Jack" variety soybean meal

Reference Articles: A commercial variety soybean meal

Test, control and the commercial soybean meal were produced under Syngenta Production Plan 10RS000001, 10RS000015 and S03-18-20-23. Processing of soybean into test, control and commercial meal evaluated in this study was conducted under Syngenta Processing Contract Agreement C09-00364 and was performed by the Food Protein Research and Development Center, Texas A&M University, College Station, TX. Information on planting and harvest dates, herbicide application, and storage will be archived at Syngenta.

Syngenta Field Production

Identification Number	Materials Description
10RS000001	Transgenic SYHT0H2 soybean
10RS000015	Nontransgenic, near-isogenic control
S03-18-20-23	Commercial reference variety

Classification: Feed ingredient

Chain-of-Custody: Syngenta provided the chain-of-custody records for each soybean meal lot delivered.

Shipping: Syngenta was responsible for shipping the test, control and reference articles and ensuring that the products were shipped in compliance with existing regulations.

Storage Requirements: Ambient temperature during shipment and upon storage at CQR, in a secure area

Method of Administration: Orally via complete feed

Frequency of Administration: *Ad libitum* for approximately 42 days starting at placement of chicks (study Day 0)

Justification: Feed was the route of administration

Preparation Before Use: Each type of soybean meal was added to the feed ingredients and was thoroughly mixed to ensure uniform dispersion.

Analyses:	Characterization of soybean meal, post processing, is reported in a Final Report issued from the processing facility, and was conducted under separate contract at the Food Protein Research and Development Center. Pre-diet manufacturing analysis of soybean meal was assessed and reported in a Certificate of Analysis (COA) from Covance Laboratories. Analyses included a pesticide profile and a nutrient / anti-nutrient analysis. Verification of identity of the test and control soybean was conducted on soybeans by event-specific PCR prior to processing and the results are archived at Syngenta. Identity of the commercial soybean meal lots was confirmed by chain-of-custody.
Accounting:	All quantities of test, control, and commercial articles (soybean meal) received, used and disposed of were documented. Excess soybean meal was disposed of according to the Sponsor's directions.

3.3 Test System

3.3.1 Justification

Soybean meal is a typical component of commercial broiler chicken diets. Due to their rapid growth, broiler chickens are sensitive to the nutritional effects of dietary components, and represent a suitable test system for assessing the nutritional quality of transgenic soybeans.

3.3.2 Specifications

One-day-old male and female Cobb × Cobb 500 chicks were obtained from Simmons Foods Hatchery for use in this study. All birds were received from the same hatchery at the same time. Birds were delivered from the hatchery via ground transportation directly to the test facility. After receipt at the test facility the chicks were examined by a veterinarian and only healthy chicks were placed in the study.

Species:	Chicken (*Gallus domesticus*)
Strain:	Commercial production broiler
Breed:	Cobb × Cobb 500
Sex:	Male and female (vent sexed at hatchery)
Supplier:	Simmons Foods Hatchery, Pineville, MO
Age:	Newly hatched chicks, approximately 1 day of age at placement (study Day 0)
Identification:	Pen cards bearing treatment number and treatment color code. Birds were individually identified with numbered wing tags prior to obtaining individual weights for yield data.
Number of birds:	360
Number of treatments:	3
Number of pens/treatment:	10 (five pens with males, five pens with females)
Number of birds/pen:	12
Number of birds/treatment:	120 (60 males/ 60 females)
Total number of pens:	30

4.0 EXPERIMENTAL DESIGN

4.1 Treatment Description

Treatments were assigned to pens using a randomized complete block design. The test facility was divided into 5 blocks of 6 pens each. Birds were assigned to the pens randomly within gender according to CQR SOP B-10. The general study design is shown in Table 1.

TABLE 1 Summary of experimental design

Treatment[1]	Soybean meal ID [2]	No. of Pens of Each Sex	No. of Males/Pen	No. of Females/Pen	Total No. of Birds/Sex	Total No. Birds/Treatment
1	SYHT0H2 Soybean Test	5	12	12	60	120
2	Nontransgenic Control	5	12	12	60	120
3	Commercial Variety	5	12	12	60	120
	Total	30			180	360

[1] Treatment identity remained blinded until the in-life phase of the study was completed.
[2] Due to blinding the identity of the soybean meal was included in the final report.

4.2 Control of Bias

The test, control, and commercial soybean meal were assigned to a specific treatment group by the Study Director. The assignment was placed in the study file and was made part of the final report. Personnel conducting day-to-day management of broilers were blinded to the treatment identification. Test, control, and commercial soybean meal were handled identically to minimize bias.

5.0 FEED AND WATER

5.1 Soybean meal - Preparation and Samples

Characterization of soybean meal used in this study was conducted under separate contract and the report is maintained by the Sponsor. The results are reported in Appendix A - Table A3. Soybean meal analyses included verification of identity of test (SYHT0H2 soybean) and control soybean by polymerase chain reaction (PCR) analyses prior to processing, and pesticide and nutrient / anti-nutrient analyses of soybean meals.

Soybean meal for this study was shipped by the Food Research and Development Center, Cater-Mattil Hall, 2476 TAMU, College Station, TX 77843-2476 (c/o Richard Clough; tel 979-862-2262; email rclough@tamu.edu) to Colorado Quality Research, Inc. (CQR) in containers suitable to maintain the identity of the different soybean meal lots. Upon receipt, CQR maintained the identity of the different soybean meal lots and handled the grain in a manner (SOP FM-2) to ensure there was no mixing among the different soybean meal lots.

Each lot of soybean meal was sampled prior to use in diet mixing according to CQR feed sampling procedures; for each lot, two representative composite sub-samples were collected. The two approximately 300-g sub-samples were labelled with the study number and soybean meal lot number. One sub-sample was sent, under ambient temperature and humidity, to the Sponsor to be retained. The second sub-sample was retained at CQR, until request of the Sponsor, at which time the second set of sub-samples were sent, on dry ice, to the Sponsor for analysis and long term storage.

The test, control and commercial soybean meal were labeled and packaged to preserve identity throughout the study. Labels included the CQR Study Number and the soybean meal identification (the same identification of the soybean meal as provided by the Sponsor).

5.2 Treatment Diets – Formulation, Preparation, and Samples

Diet treatments in this study consisted of two separate formulations, or diet types: a starter and grower/finisher diet. Each formulation used one of the soybean meal experimental materials to replace the normal soybean meal content of broiler diets. Each diet consisted predominantly of a mixture of soybean meal (either test, control or commercial soybean meal) and corn grain. For each diet type (starter (32.8-33.5%) and grower/finisher (29-30%)), the treatment diets were formulated to be isocaloric and contain approximately the same amount of soybean meal. Diets were formulated to maximize the amount of soybean meal included, while meeting the above diet specifications.

The sources of dietary protein used in this study were primarily soybean meal and corn (maize). Diets conformed as closely as possible to industry standards and/or the nutritional recommendations set forth in the publication "Nutritional Requirements of Poultry, 9th revised edition" by the National Research Council (NRC, 1994). All starter and grower/finisher diets contained salinomycin (50 g/ton) as a coccidiostat. The diets were not expected to contain any known contaminants that would interfere with the study objectives.

Treatment diets were mixed at the CQR feed mill. Vertical mixers (500-lb and 4000-lb capacity) and a California Pellet Mill system were used to prepare the diets. Feed was pelleted through an approximately 5-mm die with live steam addition. Starter diets were fed as crumbles and the grower/finisher diets were fed as pellets.

After the starter diets had been pelleted and crumbled, and grower/finisher diets had been pelleted, samples were collected as the feed flowed into bulk storage boxes. For each of the starter and grower/finisher diets, the collected sample was thoroughly mixed by hand prior to collecting three sub-samples of approximately 300 g each.

- One set of the 300 g samples was sent to Missouri of University for analyses listed in the table in Table 2.
- The second set of 300g samples was stored at CQR at < -20^{o}C, until shipped to Syngenta (Justin McDonald) approximately 2 weeks after diet preparation to be analyzed and then placed into long term storage at < -20^{o}C.

  Justin McDonald
  Syngenta Crop Protection
  3054 E Cornwallis Dr
  Durham, NC 27709, USA
- The third set of 300 g samples was retained at CQR under ambient temperature and humidity conditions until time of shipping to Syngenta for long-term storage.

5.3 Assays

Diets were assayed for the analytes listed in Table 2 below. Diets were not assayed for salinomycin (coccidiostat)

TABLE 2 Diet Analyses

Laboratory	Sample type	Analytes
Univ. of Missouri	Complete diets	Protein, amino acids, moisture, acid detergent fiber, neutral detergent fiber, crude fiber, crude fat, ash, calcium, phosphorus, potassium, sodium, chloride, and zinc.

5.3.1 Syngenta Analyses of Diets

Samples of diets and the respective soybean meal preparations from SYHT0H2 soybean, nontransgenic control soybean, and the commercial soybean varieties underwent analytical testing for transgenic protein and DNA content. The Syngenta principle investigator was notified before shipment of the samples.

Samples of the SYHT0H2 and nontransgenic soybean meal were analyzed by enzyme-linked immunosorbent assay (ELISA) for the detection and quantification of the transgenic proteins, AvHPPD-03 and PAT, associated with SYHT0H2 soybean. The commercial soybean meal was not analyzed by ELISA. The three soybean meal samples (SYHT0H2, nontransgenic control, and commercial control soybean) and the corresponding diets were analyzed by PCR to confirm the presence or absence of SYHT0H2 soybean DNA. The results of the ELISA and PCR analyses and method descriptions are documented in the analytical phase report presented in Appendix F.

5.4 Water

A copy of the CQR facility semi-annual water analysis report for total coliforms, conducted by Stewart Environmental Associates, and a copy of the most recent water analysis report from the Northern Colorado Water Association are archived with the original CQR study records. Based on the water results, the water was potable and suitable for human consumption, and therefore acceptable for use in this study.

6.0 HOUSING AND MANAGEMENT

6.1 Housing

Assignment of treatments to pens was conducted using a computer (Microsoft Office Excel 2003[1]) random numbers generator. The computer-generated assignment appears in Table 3.

TABLE 3 Treatment Assignments

	Treatment Assignment to Pens in Block - **Females**					Treatment Assignment to Pens in Block - **Males**				
Trt	1	2	3	4	5	1	2	3	4	5
1	7	11	17	25	29	6	12	18	20	30
2	5	8	15	23	31	2	9	19	22	27
3	4	13	14	21	28	3	10	16	24	26

[1] Microsoft Office Excel 2003. Copyright © 1985-2003 by Microsoft Corporation, Redmond, WA, USA.

Birds were housed within an environmentally controlled facility in concrete floor pens (approximately 4 ft × 4 ft = 1.22 m x 1.22 m) providing approximately 1.33 ft^2 per bird = 1.49 m^2 (including feeder and waterer space). Pens were constructed of solid plastic (4 ft tall = 1.22 m) in an environmentally controlled facility. Birds were placed in clean pens containing an appropriate depth of wood shavings to provide a comfortable environment for the chicks. Additional shavings were added to pens if they become too damp for comfortable conditions for the test birds during the study. Lighting was via incandescent lights and a commercial lighting program was used as shown in Table 4.

TABLE 4 Lighting

Approximate Bird Age (days)	Approximate Hours of Continuous Light Per 24-Hr Period	Approximate Light Intensity (foot candles)
0 – 4	24	1.0 – 1.3
5 – 10	10	1.0 – 1.3
11 – 18	12	0.2 – 0.3
19 – study end	16	0.2 – 0.3

Environmental conditions of floor space, temperature, lighting, bird density, feeder and drinker space were similar for all treatment groups.

In order to prevent bird migration, each pen was checked to ensure no openings greater than 1 inch (2.54 cm) existed for approximately 12 inches (30.5 cm) in height between pens. To achieve this, a solid (wood or plastic) divider was in place for approximately the first 12 inches (30.5 cm) from the floor between each pen.

6.2 Management

6.2.1 Vaccinations

Birds were vaccinated for Marek's disease at the hatchery. Birds were vaccinated at CQR for Newcastle disease and infectious bronchitis by spray application on study Day 0. The vaccine was obtained from Fort Dodge Animal Health and identified as Newcastle Bronchitis Vaccine B1 type B1 strain, Massachusetts type, live virus (lot number 1091209A, expiration date 27 May 2012). A record of the vaccination is included with the raw data for this report. No other vaccinations were administered during the study.

6.2.2 Water

Water was provided *ad libitum* throughout the study via one automatic bell drinker per pen. Drinkers were checked twice daily and cleaned as needed to ensure a clean and constant water supply to the birds.

6.2.3 Feed

Feed was provided *ad libitum* throughout the study (except for the pre-processing feed withdrawal period described in Section 7) via one hanging tube feeder per pen. A feeder tray was placed in each pen for the first 4 days of the study. Birds were placed on their respective

treatment diets upon receipt and diets were fed continuously during the study period. Feed added and removed from pens was weighed and recorded. Diet changes were conducted at the same time for all pens. The starter diet was fed from Day 0 – 21 and the grower/finisher diet was fed for the remainder of the study.

6.2.4 Daily Observations

The test facility, pens, and birds were observed at least twice daily for general flock condition, lighting, water, feed, ventilation, and unanticipated events. The minimum and maximum temperatures of the test facility were recorded once daily.

6.2.5 Mortality, Culls and Sex-slips

From study Day 0 to Day 42, any bird that was removed and euthanized or found dead was weighed and recorded on the pen mortality record. Birds that died after collection of Day 42 pen weights, but before collection of individual bird weights on Day 43 were recorded on the individual live bird weight data form as Dead Prior to Individual Weights (DPIW) and were not weighed, necropsied or listed on the pen mortality record. Birds that died after collection of individual bird weights on Day 43 were recorded as Dead on Arrival (DOA) at processing on the processing trailer documentation form and on the hot weight (weight of intact carcass immediately after euthanasia and prior to chilling) data form for clarity of bird accounting. These birds were not necropsied or listed on the pen mortality record. Cull birds (birds unable to reach feed or water, or generally unthrifty birds) were removed by technicians blinded to treatment identification. When mis-sexed birds were noted, they were removed, euthanized, weighed, and recorded on the pen mortality record. Dead birds were necropsied to the extent necessary to determine the probable cause of death. Probable cause of death and necropsy findings were recorded on the pen mortality record.

6.2.6 Body Weights and Feed Intake

Birds were weighed, by pen, on study Day 0 (receipt of chicks) and Day 42 (end of performance evaluation phase). Birds were wing tagged and individually weighed immediately prior to slaughter for processing. The feed remaining in the feeder at Day 21 and Day 42 was weighed and the amount consumed per pen was calculated by subtracting the quantity of feed remaining in the pen from the starting quantity of feed

6.2.7 Weight Gain and Feed:Gain

Performance data were calculated and summarized by average weight gain per bird on Day 42. The average feed:gain was calculated for the period from Day 0 – Day 42 by dividing the total feed consumption by the total weight gain of surviving birds for that pen. Adjusted feed:gain was calculated by dividing the total feed consumption by the weight gain of surviving birds plus the weight gain of birds that died or were removed from that pen. For example: Adjusted feed:gain Day 0 – Day 42 = Feed intake during Days 0 – Day 42 ÷ [(Day 42 pen weight – Day 0 pen weight) + (mortality/removal weights Day 0 – Day 42 – average bird weight Day 0) {this is conducted on an individual bird basis}]. If the dead or removed bird(s) lost weight, then no adjustment was made for that bird.

6.2.8 Scales

Scales used in preparation of feed and weighing of feed and birds were licensed by the State of Colorado. At each use, the scales were checked using standard weights according to CQR Standard Operating Procedures. A copy of the State scale inspection and license is archived with the original study records.

7.0 PROCESSING – YIELD DATA AND SAMPLES FOR ANALYSIS

Processing was conducted according to CQR SOP B-71. After the weight data had been collected on Day 42, the respective feed was returned to the pens. Feed was removed from the pens approximately 12 hours prior to the scheduled processing time on Day 43. All surviving birds in each pen were processed. Birds were processed by first euthanizing the bird (by complete decapitation with an electric stun knife), scalding, plucking and eviscerating. The weights of each carcass were immediately recorded after eviscerating and then each carcass was submitted for breast deboning.

7.1 Yield Data

(Includes the following data for individual birds)

- Live weight
- Carcass weight
- Breast meat weight –skinless, boneless
- Wings weight (bone in, skin on)
- Thighs weight (bone in, skin on)
- Drums weight (bone in, skin on)

Units of measure for the individual weights were either grams or kilograms as indicated on the respective data collection form. Part weights were expressed on a percentage basis, relative to total carcass weight. This was done by dividing the weight of the part into the weight of the whole carcass immediately after the animal has been euthanized, scalded, plucked and eviscerated. For example, percent breast yield = breast weight ÷ hot carcass weight × 100 percent.

8.0 STATISTICAL ANALYSIS

Statistical analyses were conducted on growth performance (body weight), carcass weight and portions, feed consumption, and feed conversion ratio (unadjusted for mortality). The pen was used as the experimental unit. Statistical significance was defined as alpha = 0.05. ANOVA was used to assess treatment-related differences. The statistical model included block, all treatments, sex, and the treatment-by-sex interaction as fixed effects. If the treatment-by-sex interaction was not significant, the main effect of treatment was evaluated. If the treatment-by-sex interaction was significant ($P \leq 0.05$), this was judged to undermine the validity of comparing treatments across genders, in which case within-sex treatment effects were assessed.

Within the model framework stated above, the significance of the specific comparison between test and the nontransgenic control treatments were determined in all cases, regardless of the significance of the overall treatment effect (two-sided test).

9.0 DISPOSITIONS

9.1 Excess Test, Control, and Reference Articles, and Duplicate Meat Samples

An accounting of soybean meal received and used was documented. Any soybean meal not used to mix the complete feed was disposed of by burial at a local commercial landfill. Soybean meal retention samples were sent to the Sponsor for archiving (were sent on dry ice).

9.2 Feed

An accounting was maintained of all treatment diets. The amounts mixed, used, and discarded were documented. Unused feed was disposed of by placing into a dumpster for commercial transport to a local landfill for burial. Feed retention samples were sent to the Sponsor (were sent on dry ice) for archiving.

9.3 Test Animals

An accounting was maintained of birds received for the study. Birds were sacrificed on Day 43 for processing (the meat from these birds was not used for human consumption). Carcasses, meat, mortalities and removed birds were transported to a commercial landfill for burial. Documentation of disposition is archived with this final study report.

9.4 Records and Report

Audited data (Excel workbook file) were sent to Steve Radecki for statistical analyses. After review of the draft reports and after the statistician's report was signed, a signed original final report, including the signed QA statement and all other information required by the GLP regulations, was prepared by the Study Director and sent to the Sponsor. Any revision to the signed report will be documented as a Report Amendment(s).

The Study Director's final study report, original data and study records, statistician's report and Sponsor's data and reports (analysis of test, control, and reference articles) are stored in the Syngenta Company Regulatory archives. An exact copy of the final report and all study records will be retained for five years at the CQR archive. The CQR archive is located at 400 East County Road 72, Wellington, Colorado.

All original data and records generated at the University of Missouri will be retained at the University of Missouri facility for a minimum of three years.

10.0 CONDUCT OF STUDY AND TEST MONITORING

This study was conducted in accordance with the study protocol, CQR Standard Operating Procedures, and the principles and guidelines for the care and use of agricultural animals in research (FASS, 2010). This study was conducted in compliance with the Food and Drug Administration's Good Laboratory Practice for Nonclinical Laboratory Studies regulation (21CFR, Part 58). The [redacted] CQR contract Quality Assurance Unit (QAU) conducted in-life phase inspections, and the study data and report were audited to ensure the integrity of the data generated by CQR. The portion of the study conducted by Syngenta was conducted in compliance with the United States Environmental Protection Agency Good Laboratory Practice Standards (40CFR, Part 160). Syngenta QAU provided oversight for data generated at CQR and Syngenta, and statistical analysis of data by Steve Radecki.

11.0 PERSONNEL

Key personnel involved in this study were as follows:

Study Monitor
Sponsor Representative
Quality Assurance
Statistician
University of MO –
feed and meat analysis
Testing Facility Management
Study Director
Operations Manager
Farmer Manager
Feed Mill Manager
Research Technician
Processing Supervisor
Consulting Nutritionist

[redacted]

12.0 RESULTS AND CONCLUSIONS

12.1 Results

The purpose of this study was to evaluate whether broiler chickens fed diets prepared with SYHT0H2 transgenic soybean meal exhibited any adverse effects on survival, feed consumption, growth, feed conversion, or carcass characteristics when directly compared with broiler chickens fed diets prepared with nontransgenic, near-isogenic soybean meal. A commercial reference soybean variety was also included in the analyses.

The results of compositional, pesticide, microbial, and mycotoxin analyses of soybean meal lots prior to diet formulation and prior to use of those diets in this study were reported by Covance, Laboratories under a separate protocol and the full report can be found in Appendix D. The results are presented in Appendix A - Tables A1 and A2. Results of event-specific PCR testing of SYHT0H2 soybean and control soybean seed lots to verify the identity of test and control articles prior to meal processing are presented in Appendix A – Table A3. Analytical results for corn grain and corn gluten meal lots used in all study diets are presented in Appendix A - Table A4.

Dietary treatment assignments for the three soybean meal lots are presented in Appendix B - Table B1. The starter and grower/finisher diet formulations and calculated nutrient composition are shown in Appendix B - Tables B2 and B3. The results of analyzing the formulated diets for the starter and grower/finisher diets (Appendix B – Tables B4 and B5, respectively) were acceptable based on a review conducted by the consulting nutritionist.

Performance data for this study are presented in Appendix C - Tables C1 and C2. Summary statistics for bird performance, processing (yield) parameters, and results of statistical analyses are presented in tabular form in Appendix C- Tables C1 through C5. The statistical analysis sub-report, including tables of selected parameter data, is appended (Appendix E).

Initial (Day 0) bird weights (12 birds placed per pen) are summarized by treatment and pen in Appendix C - Table C6. Mortality by dietary treatment ranged from 4.2 to 5.8 % (average of 5.3% across all dietary treatments; SYHT0H2 and the nontransgenic control fed birds had

equal mortality (n=7) (Appendix C - Table C7). The apparent cause of death identified at necropsy for most birds that died was bacterial infection, ascites and sudden death syndrome; these occur commonly in chickens. The birds in all groups were in good health based on twice daily pen observations.

Pen data including live weight (kg/pen) determined on Day 0 and Day 42, and pen feed consumption (starter diet from Day 0 – Day 21 and grower/finisher diet from Day 21 – Day 42) were evaluated directly and used to calculate the set of performance parameters on the specific study days or for the intervals indicated in Table 5. The interval listed as "at processing" is equivalent to "hot weight" as it indicates that measurements on carcass were taken at the time immediately after euthanizing of the birds.

The AvHPPD-03 and PAT transgenic proteins associated with SYHT0H2 soybean were not detected in the SYHT0H2 soybean meal or the nontransgenic, near-isogenic control soybean meal. However, PCR analyses confirmed the presence of SYHT0H2-soybean-specific DNA in the test soybean meal and corresponding diets, and confirmed the absence of SYHT0H2 DNA in the nontransgenic control, commercial control soybean meal, and corresponding control diets (Appendix F).

TABLE 5 Broiler Weight at Day 0, 42 and 0-42 Time Intervals

Parameter	Times or Intervals
Performance	
Avg Bird Wt. (g/bird)	Day 0
Avg Bird Wt. (kg/bird)	Day 42
Feed Intake (kg/bird)	Day 0-42
Avg Bird Gain (kg)	Day 0-42
Feed:Gain (kg/kg)	Day 0-42
Adjusted Feed:Gain (kg/kg)	Day 0-42
Carcass Yield	
Processing Live Wt. (kg/bird)	Day 43
Carcass Wt. (kg and % live wt.)	At processing
Breast Wt. (kg and % carcass hot wt.)	At processing
Drum Wt. (kg and % carcass hot wt.)	At processing
Thigh Wt. (kg and % carcass hot wt.)	At processing
Wing Wt. (kg and % carcass hot wt.)	At processing

12.1.1 SYHT0H2 Soybean Performance Parameters

The summary of the ANOVA for the performance outcomes is provided in Appendix C – Tables C1 and C2 and Appendix E – Tables 1 and 2. No statistically significant differences associated with treatment groups were observed ($P > 0.05$, Appendix C – Table C1 and Appendix E -Table 1). Additionally, pairwise comparisons of the SYHT0H2 soybean test and nontransgenic control treatment groups revealed no statistically significant differences (Appendix C and Appendix E -Table 2); all least squared means are listed for each treatment. The pairwise comparisons between each diet treatment show the results of a statistical comparison between each treatment: SYHT0H2 versus nontransgenic control (Jack-Control); SYHT0H2 versus commercial reference; nontransgenic control (Jack-Control) versus commercial reference. All pairwise comparisons evaluated for significance at $P \leq 0.05$.

There were no biologically relevant differences in broiler performance or carcass yield between broilers fed diets containing soybean meal produced from SYHT0H2 soybean and those fed diets containing commercial variety or nontransgenic control soybean meal. Performance over the 42-day test period for broilers fed diets containing SYHT0H2 soybean meal was not statistically different than that of broilers fed diets formulated with commercial soybean meal or with the nontransgenic, near-isogenic control soybean (Appendix C – Tables C1 and C2) (all values for significance were $P > 0.05$). No treatment-by-sex interactions were detected ($P > 0.05$) for performance parameters across the three treatment groups, test, control or commercial soybean meal. Measures of broiler performance were of similar magnitude for broilers fed diets formulated to the same nutrient specifications with the soybean meal component of the diet provided by SYHT0H2, nontransgenic control, or commercial soybean meal (Appendix C – Tables C1 and C2). Statistical analyses did include an evaluation of the effect of bird sex on performance measurements and all measurements were significant. These results are as expected given the natural differences between male and female bird physiology.

No unexpected effects on broiler parameters were observed when broilers were fed diets containing soybean meal produced from SYHT0H2 soybean compared to diets containing nontransgenic control or commercial soybean meal.

12.1.2 SYHT0H2 Soybean Carcass Measurements

The summary of the ANOVA for the carcass outcomes is provided in Appendix C and E – Tables E3, E4 and E5. The treatment-by-sex interaction was significant for thigh weight (Appendix C-Table C3). Further evaluation of the treatment effect within sex indicated that thigh weights of male broilers fed diets containing SYHT0H2 soybean meal did not differ significantly from thigh weights of broilers fed nontransgenic control soybean meal. Thigh weights for broilers fed the commercial variety soybean diets were different from thigh weights of broilers fed SYHT0H2 soybean diets; the commercial reference treatment resulted in a marginally greater thigh weight (Appendix C-Table C4). No treatment effect was detected for females (Appendix C – Table C4) with regard to thigh weights. No statistically significant differences associated with treatment -by-sex interactions were observed for the other carcass measurements ($P > 0.05$, Appendix C-Table C3).

An across-treatment statistical difference was noted for the breast percent of live weight parameter (Appendix C–Table C3). This difference was due to a slight decrease in this parameter for the commercial reference treatment group (Appendix C–Table C5). Feeding commercial reference diets to birds resulted in a marginally lower percent breast carcass weight compared to the nontransgenic control treatment group; a pairwise statistical comparison of all three treatments (as indicated above for performance parameters) showed that the commercial reference variety was only different from the nontransgenic control fed birds (Appendix C, Table C5). The pairwise comparison of diet treatment also revealed a gain in weight for the commercial reference fed birds compared with the nontransgenic control (Appendix C, Table C5) for the drum percent of live weight parameter. There was no significant difference between SYHT0H2 soybean meal-fed birds compared to birds fed nontransgenic control soybean meal.

Statistical analyses did include an evaluation of the effect of bird sex on carcass measurements and all measurements were significant except hot weight percent and wing percent. These results are as expected given natural differences between male and female bird anatomy.

In summary, carcass measurements were of similar magnitude for birds fed diets formulated to the same nutrient specifications with the included soybean meal component of the diet provided by SYHT0H2 soybean seed, nontransgenic control, or commercial soybean material (Appendix C –Tables C3, C4 and C5).

12.2 Conclusions

A 42-day broiler feeding study evaluated whether standard poultry diets prepared with SYHT0H2 transgenic soybean meal had an effect on male and female broiler chickens. There were no biologically relevant differences in broiler performance or carcass yield between broilers fed diets containing soybean meal produced from SYHT0H2 soybean and those fed diets containing meal from a nontransgenic, near-isogenic control soybean variety. Although there were three parameters that differed for the commercial reference variety there were no statistical differences specific for SYHT0H2 soybean meal-fed birds compared with the nontransgenic, near isogenic control meal-fed birds. The diets containing soybean meal produced from SYHT0H2 soybean were as wholesome as the diets containing nontransgenic, near-isogenic control or commercial soybean meal regarding their ability to support the rapid growth of broiler chickens.

These data support the conclusion that soybean meal produced from SYHT0H2 soybean seed is as nutritious as soybean meal from a conventional nontransgenic, near-isogenic soybean variety.

13.0 REFERENCES

FASS. 2010. Guide for the care and use of agricultural animals in research and teaching, Third edn., Federation of Animal Science Socities.

NRC. 1994. National Research Council. Nutrient Requirements of Poultry, Ninth Revised edn., National Academy Press, Washington, D.C.

Soyatech. 2012. Pages 344-349 in Soya & Oilseed Bluebook. P. Goldbitz, (ed.) Soyatech, LLC, Bar Harbor, ME.

APPENDICES SECTON

APPENDIX A Pre-study Data from Syngenta Study No. TK0036575

TABLE A1 Soybean meal compositional analyses - including pesticides (as-is basis)

Soybean Meal ID	SYHT0H2 Test	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Proximate (%)			
Moisture	5.64	6.16	6.21
Protein	44.2	46.0	51.4
Total Fat	0.794	0.519	0.659
Ash	6.70	6.99	5.84
Carbohydrates	42.7	40.3	35.9
Crude Fiber (%)	3.43	3.01	4.41
Minerals (ppm)			
Copper	14.9	17.1	17.6
Iron	137	122	102
Manganese	30.2	31.1	26.8
Zinc	81.2	75.3	50.5
Minerals (%)			
Calcium	0.177	0.205	0.251
Magnesium	0.284	0.276	0.274
Phosphorus	1.08	1.03	0.714
Potassium	2.34	2.45	2.24
Sodium	< 0.0100	< 0.0100	< 0.0100
Chloride (%)	0.0354	0.0349	0.0356
Sulfur (%)	0.454	0.453	0.434
Starch (%)	3.67	2.03	0.892
Amino Acids (%)			
Aspartic Acid	4.80	5.06	5.73
Threonine	1.75	1.83	1.95
Glutamic Acid	7.14	7.67	9.06
Proline	2.14	2.26	2.62
Glycine	1.81	1.92	2.12
Alanine	1.88	2.00	2.21
Cystine	0.710	0.696	0.701
Valine	2.09	2.19	2.39
Methionine	0.605	0.620	0.666
Isoleucine	2.02	2.11	2.39
Leucine	3.18	3.36	3.74
Lysine	2.70	2.79	3.13

TABLE A2 Soybean meal mycotoxin analyses1 (as-is basis)

Soybean Meal ID	SYHT0H2 Test	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Mycotoxins			
Aflatoxin B1 (ppb)	< 0.500	< 0.500	< 0.500
Aflatoxin B2 (ppb)	< 0.500	< 0.500	< 0.500
Aflatoxin G1 (ppb)	< 0.500	< 0.500	< 0.500
Aflatoxin G2 (ppb)	< 0.500	< 0.500	< 0.500
Total Aflatoxins (ppb)	< 2.00	< 2.00	< 2.00
T2 Toxin (ppm)	< 0.2	< 0.2	< 0.2
Deoxynivalenol (DON) (ppm)	< 0.6	< 0.6	< 0.6
Zearalenone (ppm)	< 51.7	< 51.7	< 51.7
Fumonisin B1 (ppm)	< 0.1	< 0.1	< 0.1
Fumonisin B2 (ppm)	< 0.1	< 0.1	< 0.1
Fumonisin B3 (ppm)	< 0.1	< 0.1	< 0.1
Total Fumonisins (ppm)	< 0.3	< 0.3	< 0.3

[1]Mycotoxin analyses are reported on Covance 8255-924.and are archived under the respective COA number.

TABLE A3 Verification of identity of test and control soybean prior to processing

syngenta

STEWARDSHIP QUALITY CONTROL STATEMENT
December 21, 2010

Representative seed samples of the Material lots listed below were submitted for Stewardship Quality Control (SQC) testing using Taqman® real-time polymerase chain reaction (PCR) methods. This analysis was conducted to test for Adventitious Presence (AP) of USDA deregulated events where testing methodology is available and regulated events under development in Syngenta.

MAT ID	Description	SQC Project ID	Results
10RS000001	SYHT0H2	SQC-SB-10-0297	Event (SYHT0H2) confirmed; No AP detected
10RS000002	Jack	SQC-SB-10-0297	No AP detected

[redacted]
Seeds R&D Quality Control Manager
Global Quality Management

syngenta

Syngenta Biotechnology, Inc.
3054 E. Cornwallis Rd
Research Triangle Park, NC
27709
USA

phone +001 919 226 7417
mobile +001 919 649 3010

TABLE A4 Corn grain and corn gluten meal analyses (as-is basis)

	Corn Grain	Corn Gluten Meal
Crude Protein	7.82	61.51
Moisture	12.90	10.32
Crude Fat	3.40	2.13
Amino Acids (g/100g of sample)		
Taurine	0.04	0.02
Hydroxyproline	0.01	0.00
Aspartic Acid	0.53	3.76
Threonine	0.28	2.05
Serine	0.35	2.74
Glutamic Acid	1.54	12.65
Proline	0.67	5.69
Lanthionine	0.00	0.00
Glycine	0.31	1.85
Alanine	0.58	5.37
Cysteine	0.17	1.12
Valine	0.39	2.87
Methionine	0.16	1.70
Isoleucine	0.28	2.56
Leucine	0.94	10.19
Tyrosine	0.24	3.29
Phenylalanine	0.38	3.91
Hydroxylysine	0.02	0.04
Ornithine	0.00	0.07
Lysine	0.25	1.16
Histidine	0.23	1.27
Arginine	0.36	2.10
Tryptophan	0.06	0.41

APPENDIX B Diet Composition and Analyses

TABLE B1 Treatment assignment of soybean meal lots

Treatment Number	Treatment Type[1]	Formulation Number	Soybean meal ID
Starter			
1	T	5208	SYHT0H2 Soybean
2	C	5209	Nontransgenic- Jack
3	R	5207	Commercial material
Grower/Finisher			
1	T	5208	SYHT0H2 Soybean
2	C	5209	Nontransgenic- Jack
3	R	5207	Commercial material

[1] T = test, C = control, and R = reference

TABLE B2 Starter diet formulation and calculated nutrient composition (as-is basis)

Treatment Number	**1**	**2**	**3**
Soybean Meal ID	SYHT0H2 Soybean Test	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Ingredient	**Percent of Each Ingredient**		
Corn	56.705	57.812	61.362
Soybean Meal	33.500	33.500	32.882
Corn Gluten Meal	3.997	2.876	
Soybean Oil	2.132	2.182	2.210
Defluorinated Phosphate	1.527	1.562	1.771
Limestone	1.001	0.946	0.729
Salt	0.355	0.351	0.327
DL-Methionine	0.231	0.243	0.281
L-Lysine	0.162	0.136	0.047
Choline Chloride	0.150	0.150	0.150
Poultry Vit[1]	0.100	0.100	0.100
Poultry TM P[2]	0.100	0.100	0.100
Salinomycin	0.041	0.041	0.041

[1] Vitamin premix (DSM Nutritional Products, Inc., Parsippany, NJ) provided the following per kilogram of diet: vitamin A, 9350 IU from all trans-retinyl acetate; cholecalciferol D3, 3025 IU; vitamin E, 27.5 IU from dl-α-tocopherol; vitamin B12, 13.75 µg; riboflavin, 7.7 mg; niacin, 49.5 mg; pantothenic acid, 12.1 mg; menadione, 1.925 mg; folic acid, 0.99 mg; ethoxyquin, 77 mg; biotin, 0.088 mg; thiamine, 1.925 mg, and pyridoxine, 3.08 mg.

[2] Trace mineral premix (SEM Minerals, Quincy, IL) contained 5-6% calcium and provided the following in milligrams per kilogram of diet: Mn, 120; Zn, 100; Fe, 40; Cu, 10; I, 1.4; Se, 0.3, and Mg, 26.

TABLE B2 Starter diet formulation and calculated nutrient composition (as-is basis) (Continued)

Treatment Number	**1**	**2**	**3**
Soybean Meal ID	SYHT0H2 Soybean Test	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Calculated Nutrient Composition			
Calculated ME, (Kcal/kg)[1]	1400	1400	1400
Moisture, %	9.675	9.876	10.016
Crude Protein, %	21.7	21.7	21.7
Crude Fat, %	4.368	4.339	4.469
Crude Fiber, %	2.326	2.179	2.616
Ash, %	5.762	5.83	5.371
Calcium, %	0.95	0.95	0.95
Phosphorus (total), %	0.787	0.774	0.695
Phosphorus (avail.), %	0.45	0.45	0.45
Sodium, %	0.22	0.22	0.22
Chloride, %	0.256	0.254	0.239
Potassium, %	0.991	1.026	0.941
Arginine, %	1.509	1.492	1.429
Methionine, %	0.59	0.59	0.595
Meth & Cystine, %	0.379	0.364	0.335
TSAA , %	0.969	0.954	0.93
Lysine, %	1.22	1.22	1.22
Tryptophan, %	0.286	0.284	0.273
Threonine, %	0.827	0.834	0.813
Isoleucine, %	0.938	0.942	0.958
Valine, %	1.036	1.042	1.025
Leucine, %	2.006	1.962	1.807
Glycine, %	0.856	0.876	0.887
Choline, mg/lb	1112.997	1107.323	1088.28
Manganese, ppm	138.98	139.211	137.503
Zinc, ppm	139.854	137.655	128.657
Copper, ppm	18.311	18.828	18.351
Iron, ppm	259.405	253.248	253.779
Iodine ,ppm	1.4	1.4	1.4
Selenium, ppm	0.429	0.419	0.393
Molybdenum, ppm	0.863	0.859	0.845
Magnesium, %	0.149	0.146	0.142
Sulfur, %	0.217	0.212	0.192

TABLE B3 Grower/Finisher diet formulation and calculated nutrient composition (as-is basis)

Treatment Number	**1**	**2**	**3**
Soybean Meal ID	SYHT0H2 Soybean Test	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Ingredient	**Percent of Each Ingredient**		
Corn	60.804	61.796	65.141
Soybean Meal	30.000	30.000	29.000
Corn Gluten Meal	3.227	2.223	
Soybean Oil	2.481	2.526	2.462
Defluorinated Phosphate	1.415	1.447	1.635
Limestone	0.982	0.932	0.751
Salt	0.367	0.364	0.342
DL-Methionine	0.219	0.230	0.255
L-Lysine	0.153	0.130	0.063
Choline Chloride	0.110	0.110	0.110
Poultry Vit [1]	0.100	0.100	0.100
Poultry TM P[2]	0.100	0.100	0.100
Salinomycin	0.041	0.041	0.041

[1] Vitamin premix (DSM Nutritional Products, Inc., Parsippany, NJ) provided the following per kilogram of diet: vitamin A, 9350 IU from all trans-retinyl acetate; cholecalciferol D3, 3025 IU; vitamin E, 27.5 IU from dl-α-tocopherol; vitamin B12, 13.75 µg; riboflavin, 7.7 mg; niacin, 49.5 mg; pantothenic acid, 12.1 mg; menadione, 1.925 mg; folic acid, 0.99 mg; ethoxyquin, 77 mg; biotin, 0.088 mg; thiamine, 1.925 mg, and pyridoxine, 3.08 mg.

[2] Trace mineral premix (SEM Minerals, Quincy, IL) contained 5-6% calcium and provided the following in milligrams per kilogram of diet: Mn, 120; Zn, 100; Fe, 40; Cu, 10; I, 1.4; Se, 0.3, and Mg, 26.

TABLE B3 Grower/Finisher diet formulation and calculated nutrient composition (as-is basis) (Continued)

Treatment Number	**1**	**2**	**3**
Soybean Meal ID	SYHT0H2 Soybean Test	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Calculated Nutrient Composition			
Calculated ME, (Kcal/kg)[1]	1425	1425	1425
Moisture, %	9.928	10.108	10.262
Crude Protein, %	20	20	20
Crude Fat, %	4.806	4.78	4.819
Crude Fiber, %	2.265	2.133	2.517
Ash, %	5.442	5.503	5.087
Calcium, %	0.9	0.9	0.904
Phosphorus (total), %	0.735	0.723	0.651
Phosphorus (avail.), %	0.42	0.42	0.42
Sodium, %	0.22	0.22	0.22
Chloride, %	0.264	0.262	0.249
Potassium, %	0.919	0.951	0.866
Arginine, %	1.385	1.369	1.305
Methionine, %	0.55	0.55	0.55
Meth & Cystine, %	0.353	0.339	0.314
TSAA , %	0.903	0.889	0.864
Lysine, %	1.12	1.12	1.12
Tryptophan, %	0.262	0.26	0.249
Threonine, %	0.761	0.768	0.748
Isoleucine, %	0.859	0.863	0.875
Valine, %	0.957	0.962	0.947
Leucine, %	1.854	1.815	1.697
Glycine, %	0.791	0.809	0.817
Choline, mg/lb	985.895	980.814	962.303
Manganese, ppm	137.778	137.985	136.396
Zinc, ppm	137.467	135.498	127.393
Copper, ppm	17.71	18.173	17.753
Iron, ppm	242.223	236.71	238.35
Iodine ,ppm	1.4	1.4	1.4
Selenium, ppm	0.42	0.411	0.391
Molybdenum, ppm	0.83	0.826	0.811
Magnesium, %	0.141	0.138	0.134
Sulfur, %	0.201	0.196	0.178

[Kcal/lb × 2.2 = Kcal/kg][1]

TABLE B4 Analyzed nutrient composition of starter diets (as-is basis)

Treatment Number	**1**	**2**	**3**
Soybean Meal ID	SYHT0H2 Soybean Test	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Assay Component			
Proximates			
Crude Protein, %	22.33	22.36	22.02
Moisture, %	9.73	9.68	10.11
Crude Fat, %	4.40	4.18	4.31
Crude Fiber, %	1.36	1.30	1.70
Ash, %	5.92	5.85	5.42
Acid detergent fiber, %	2.55	2.37	2.74
Neutral detergent fiber, %	7.77	7.43	7.74
Minerals			
Calcium, %	1.13	1.12	1.06
Phosphorus,%	0.97	0.96	0.84
Sodium, %	0.26	0.26	0.24
Potassium, %	1.23	1.29	1.17
Chloride, %	0.28	0.31	0.22
Zinc (ppm)	150	149	136

TABLE B4 Analyzed nutrient composition of starter diets (as-is basis) (Continued)

Treatment Number	**1**	**2**	**3**
Soybean Meal ID	SYHT0H2 Soybean Tes	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Treatment Number	**1**	**2**	**3**
Amino Acids (g/100g of sample)			
Taurine	0.01	0.01	0.01
Hydroxyproline	0.00	0.00	0.00
Aspartic Acid	2.14	2.19	2.26
Threonine	0.77	0.78	0.77
Serine	0.84	0.86	0.89
Glutamic Acid	3.81	3.86	3.91
Proline	1.30	1.30	1.25
Lanthionine	0.04	0.04	0.04
Glycine	0.88	0.89	0.89
Alanine	1.19	1.17	1.07
Cysteine	0.38	0.38	0.34
Valine	1.11	1.11	1.07
Methionine	0.57	0.57	0.57
Isoleucine	0.98	0.98	0.96
Leucine	2.10	2.06	1.90
Tyrosine	0.76	0.77	0.73
Phenylalanine	1.11	1.11	1.09
Hydroxylysine	0.02	0.02	0.02
Ornithine	0.01	0.01	0.01
Lysine	1.26	1.28	1.24
Histidine	0.58	0.58	0.57
Arginine	1.34	1.38	1.46
Tryptophan	0.25	0.26	0.26

TABLE B5 Analyzed nutrient composition of grower/finisher diets (as-is basis)

Treatment Number	**1**	**2**	**3**
Soybean Meal ID	SYHT0H2 Soybean Test	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Assay Component			
Proximates			
Crude Protein, %	20.74	20.70	20.66
Moisture, %	9.53	9.95	10.06
Crude Fat, %	4.86	4.62	4.63
Crude Fiber, %	1.51	1.29	1.64
Ash, %	5.62	5.73	5.47
Acid detergent fiber, %	2.57	2.33	2.76
Neutral detergent fiber, %	8.43	7.32	8.23
Minerals			
Calcium, %	1.08	1.10	1.12
Phosphorus,%	0.90	0.90	0.83
Sodium, %	0.28	0.25	0.28
Potassium, %	1.15	1.18	1.12
Chloride, %	0.30	0.29	0.29
Zinc (ppm)	155	159	175

TABLE B5 Analyzed nutrient composition of grower/finisher diets (as-is basis) (Continued)

Treatment Number	**1**	**2**	**3**
Soybean Meal ID	SYHT0H2 Soybean Test	Jack Control	Commercial Control
Description	**Test**	**Control**	**Reference**
Treatment Number	**1**	**2**	**3**
Amino Acids (g/100g of sample)			
Taurine	0.01	0.01	0.01
Hydroxyproline	0.00	0.00	0.00
Aspartic Acid	1.97	2.02	2.10
Threonine	0.73	0.73	0.74
Serine	0.83	0.82	0.92
Glutamic Acid	3.53	3.58	3.65
Proline	1.23	1.22	1.19
Lanthionine	0.04	0.04	0.04
Glycine	0.81	0.83	0.85
Alanine	1.10	1.09	1.02
Cysteine	0.36	0.36	0.32
Valine	1.00	1.02	0.98
Methionine	0.55	0.55	0.53
Isoleucine	0.88	0.90	0.89
Leucine	1.93	1.91	1.80
Tyrosine	0.71	0.72	0.70
Phenylalanine	1.02	1.02	1.02
Hydroxylysine	0.02	0.02	0.02
Ornithine	0.01	0.01	0.02
Lysine	1.16	1.18	1.16
Histidine	0.53	0.54	0.54
Arginine	1.24	1.27	1.36
Tryptophan	0.25	0.25	0.24

APPENDIX C Bird Performance and Processing Data

TABLE C1 Summary of the ANOVA for Day 42 performance weight gain and feed to gain ratio

Variable	P-values			
	Block	Treatment	Sex	Treatment x Sex interaction
Day 42 body weight (pen basis)	0.6379	0.2079	0.0009	0.3374
Gain per bird (Day 0 - 42)	0.0758	0.6246	<.0001	0.5736
Gain per pen (Day 0 - 42)	0.6337	0.2095	0.0009	0.3392
Feed Intake (pen basis)	0.5621	0.4113	0.0010	0.5607
Feed to gain ratio (unadjusted for mortality)	0.3837	0.1247	0.0121	0.2451
Feed to gain ratio (adjusted for mortality)	0.2821	0.4599	<.0001	0.2234
Gain per pen (adjusted for mortality)	0.6639	0.3535	<.0001	0.4757

TABLE C2 Least squares means for Day 42 performance weight gain and feed to gain ratio

Variable	SYHT0H2-Test	Jack-Control	Commercial-Reference	SEM
Day 42 body weight, kg (pen basis)	31.02	33.17	33.11	0.9401
Gain per bird, kg (Day 0 - 42)	2.89	2.92	2.92	0.0230
Gain per pen, kg (Day 0 - 42)	30.53	32.68	32.63	0.9409
Feed Intake, kg (pen basis)	50.77	52.29	51.70	0.7978
Feed to gain ratio (unadjusted for mortality)	1.68	1.61	1.59	0.0297
Feed to gain ratio (adjusted for mortality)	1.53	1.54	1.53	0.0054
Gain per pen, kg (adjusted for mortality)	33.15	34.11	33.92	0.4845

TABLE C3 Summary of the ANOVA for carcass measurements-P-values

Variable	P-values			
	Block	Treatment	Sex	Treatment x Sex interaction
Breast Weight	0.3102	0.2236	<.0001	0.7802
Breast percent	0.1992	**0.0438**	<.0001	0.7027
Drum Weight	0.0062	0.2387	<.0001	0.5480
Drum percent	0.0399	0.0641	<.0001	0.9402
Live Weight	0.1370	0.8219	<.0001	0.2762
Thigh Weight	0.3092	0.5357	<.0001	**0.0466**
Thigh percent	0.8829	0.2468	0.0109	0.1884
Wing Weight	0.2503	0.9121	<.0001	0.1394
Wing percent	0.7242	0.2199	0.6571	0.3036
Hot percent*	0.7355	0.2453	0.0930	0.1661
Hot Weight	0.2163	0.5364	<.0001	0.5938

P-value bolded are significantly different at P < 0.05 or equivalent

*All values were calculated as "percent of live weight"

TABLE C4 Least squares means for carcass outcomes - sex * treatment

Variable	Sex	SYHT0H2-Test	Jack-Control	Commercial-Reference	SEM
Thigh Weight	F	0.31	0.31	0.31	0.004
	M	0.36a	0.37ab	0.38b	0.004

a,b: values with no common letter are significantly different at P < 0.05

TABLE C5 Least squares means for carcass measurements

Variable	SYHT0H2-Test	Jack-Control	Commercial-Reference	SEM
Breast percent of live weight	32.52%ab	32.91%a	32.15%b	0.20%
Breast weight (Kg)	0.66	0.68	0.66	0.009
Drum percent of live weight	12.48%ab	12.45%a	12.68%b	0.07%
Drum weight (Kg)	0.26	0.26	0.26	0.002
Live weight (Kg)	2.83	2.84	2.86	0.030
Thigh weight (Kg)	0.34	0.34	0.34	0.003
Thigh percent of live weight	16.55%	16.31%	16.62%	0.13%
Wing percent of live weight	10.13%	9.94%	10.01%	0.08%
Wing weight (Kg)	0.21	0.21	0.21	0.002
Hot Carcass percent of Live wt	71.94%	72.94%	71.96%	0.47%
Hot Carcass weight (Kg)	2.04	2.07	2.06	0.019

a,b: values with no common letter are significantly different at $P < 0.05$

TABLE C6 Day 0 body weights (02/01/12)

Treatment Number	Soybean Meal ID	Sex	Pen	Day 0 Number of Birds Weighed	Day 0 Pen Wt. (kg)	Day 0 Avg Bird Wt (kg)	Treatment Number	Soybean Meal ID	Sex	Pen	Day 0 Number of Birds Weighed	Day 0 Pen Wt. (kg)	Day 0 Avg Bird Wt (kg)
1	SYHT0H2 Soybean	f	7	12	0.488	0.041	1	SYHT0H2 Soybean	m	6	12	0.490	0.041
1		f	11	12	0.486	0.041	1		m	12	12	0.484	0.040
1		f	17	12	0.482	0.040	1		m	18	12	0.474	0.040
1		f	25	12	0.470	0.039	1		m	20	12	0.460	0.038
1		f	29	12	0.498	0.042	1		m	30	12	0.484	0.040
Total & Average				60	0.485	0.040	Total & Average				60	0.478	0.040
Standard Deviation					0.0102	0.0008	Standard Deviation					0.0118	0.0010
CV					2.1%	2.1%	CV					2.5%	2.5%
2	Jack	f	5	12	0.490	0.041	2	Jack	m	2	12	0.482	0.040
2		f	8	12	0.466	0.039	2		m	9	12	0.494	0.041
2		f	15	12	0.490	0.041	2		m	19	12	0.484	0.040
2		f	23	12	0.502	0.042	2		m	22	12	0.478	0.040
2		f	31	12	0.490	0.041	2		m	27	12	0.486	0.041
Total & Average				60	0.488	0.041	Total & Average				60	0.485	0.040
Standard Deviation					0.0131	0.0011	Standard Deviation					0.0059	0.0005
CV					2.7%	2.7%	CV					1.2%	1.2%
3	Commercial	f	4	12	0.492	0.041	3	Commercial	m	3	12	0.484	0.040
3		f	13	12	0.486	0.041	3		m	10	12	0.484	0.040
3		f	14	12	0.472	0.039	3		m	16	12	0.486	0.041
3		f	21	12	0.460	0.038	3		m	24	12	0.486	0.041
3		f	28	12	0.502	0.042	3		m	26	12	0.496	0.041
Total & Average				60	0.482	0.040	Total & Average				60	0.487	0.041
Standard Deviation					0.0166	0.0014	Standard Deviation					0.0050	0.0004
CV					3.4%	3.4%	CV					1.0%	1.0%

TABLE C7 Summary of mortality, removal and probable cause of death (Day 0 – 42)

Treatment		Sex	Pen No.	No. Birds Started	Number of Birds (day 0 - 42)				
					Removed[1]	Reason	Mortality	Percent	Cause of Death[2]
1	Soybean SYHT0H2	f	7	12	1	1CD/ACT		0.0%	
1		f	11	12	1	1CD/SS		0.0%	
1		f	17	12	1	1CD/BL		0.0%	
1		f	25	12				0.0%	
1		f	29	12	1	1CD/SS	1	8.3%	1ACT
1		m	6	12			2	16.7%	1BAC, 1ACT
1		m	12	12	2	1CD/PC 1CD/ACT	2	16.7%	2ACT
1		m	18	12			2	16.7%	2ACT
1		m	20	12				0.0%	
1		m	30	12	1	1CD/ACT		0.0%	
Total & Average				**120**	7		7	5.8%	
2	Jack	f	5	12			1	8.3%	1ACT
2		f	8	12				0.0%	
2		f	15	12			1	8.3%	1ACT
2		f	23	12			1	8.3%	1ACT
2		f	31	12	1	1CD/ACT	1	8.3%	1ACT
2		m	2	12			1	8.3%	1SDS
2		m	9	12				0.0%	
2		m	19	12				0.0%	
2		m	22	12			1	8.3%	1SDS
2		m	27	12			1	8.3%	1ACT
Total & Average				**120**	1		7	5.8%	
3	Commercial	f	4	12	1	1CD/BL		0.0%	
3		f	13	12			1	8.3%	1SDS
3		f	14	12	1	1CD/SS		0.0%	
3		f	21	12				0.0%	
3		f	28	12	1	1CD/SS		0.0%	
3		m	3	12			2	16.7%	1SDS 1ACT
3		m	10	12			1	8.3%	1BAC
3		m	16	12				0.0%	
3		m	24	12				0.0%	
3		m	26	12			1	8.3%	1ACT
Total & Average				**120**	3		5	4.2%	

[1] Removed = removed birds were euthanized by cervical dislocation [2]Codes: DH = dehydrated, SDS = Sudden Death Syndrome, BAC = bacterial, ACT = ascites, C = cull, SS = sex slip, BL = bad leg, ACT-S = Ascites + SDS, CD = cervical dislocation, FHN = femoral head necrosis

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Final Report

Study Title	Analysis of Nutritional Components and Environmental Contaminants in Soybean Meal for Use in Experimental Feeding Studies
Sponsor	Colorado Quality Research 400 East County Road 72 Wellington, CO 80549
Sponsor Representative	[redacted]
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Blvd Madison, WI 53704
Study Director	[redacted]
Sponsor Study No.	SYN-11-1
Covance Study No.	8255-924
Report Issued	28 June 2012
Page Number	1 of 21

TABLE OF CONTENTS

COMPLIANCE STATEMENT

This study was conducted in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards (GLPs), 40 CFR 160, with the following exceptions:

- Reference standards were not listed in the protocol but are listed in the report, will not be characterized according to GLP standards, and no reserve samples were retained from each batch.

- The stabilities of the test, control and reference substances were not verified analytically; however, that is not within the stated purpose of the study as defined by the protocol.

- T2 toxin, deoxynivalenol, zearalenone, and fumonisin analyses was performed by Romer Labs, a non-GLP facility.

- There was no approved written protocol in place at the time of sample receipt at Covance. The Sponsor provided sample storage instructions on the day of receipt. Sample receipt and storage were documented according to Covance standard operating procedures (SOPs). No further action was taken prior to protocol approval.

These exceptions had no effect on the integrity or quality of the study.

[redacted] 28 Jun 12

Study Director
Covance Laboratories Inc.

Date

Sponsor

Date

Applicant

Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc. and accurately reflects the raw data. The following study specific inspections were conducted and findings reported to the study director (SD) and associated management.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
27 Oct 2011	27 Oct 2011	Protocol Review-Madison	27 Oct 2011
31 Oct 2011	31 Oct 2011	Analytical Chemistry	01 Nov 2011
28 Nov 2011	01 Dec 2011	Draft Report and Data Review	01 Dec 2011
22 Dec 2011	22 Dec 2011	Revised Draft Report Review	22 Dec 2011
28 Dec 2011	28 Dec 2011	Protocol Amendment Review-Madison	28 Dec 2011
20 Jun 2012	20 Jun 2012	Protocol Amendment Review-Madison	20 Jun 2012
25 Jun 2012	25 Jun 2012	Revised Draft Report Review	25 Jun 2012

[redacted] 28 Jun 12

Representative
Quality Assurance Unit

Date

SIGNATURE

[redacted] 28Jun12

Study Director
Covance Laboratories Inc.

Date

COVANCE KEY PERSONNEL

Nutritional Chemistry and Food Safety

Vice President and General Manager

[redacted]

Directors

[redacted]

Associate Director

[redacted]

Study Director

[redacted]

Study Coordinator

[redacted]

Quality Assurance Senior Manager

[redacted]

Managers

[redacted]

Supervisors

[redacted]

STUDY IDENTIFICATION

Study Title:
Analysis of Nutritional Components and Environmental Contaminants in Soybean Meal for Use in Experimental Feeding Studies

Sponsor:
Colorado Quality Research, Inc.
400 East County Road 72
Wellington, CO 80549

Sponsor Representative:
[redacted]
Colorado Quality Research, Inc.
400 East County Road 72
Wellington, CO 80549
[redacted]

Sub-Contractor Laboratory: (T2 toxin, deoxynivalenol, zearalenone, fumonisin analyses)
[redacted]
Romer Labs
1301 Stylemaster Drive
Union, MO 63084-1156
[redacted]

Compositional Analysis Testing Facility:
Covance Laboratories Inc.
3301 Kinsman Blvd.
Madison, WI 53704

Study Director:
[redacted]
Covance Laboratories Inc.
[redacted]

Compositional Analysis QAU Contact:
[redacted]
Senior Manager
[redacted]

Analytical Study Timetable:
Analytical Study Initiation Date: 27 October 2011
Analytical Report Completion Date: 28 June 2012

INTRODUCTION

Purpose
The purpose of this study was to conduct a compositional and contaminant analysis of soybean meal processed from soybean seed. Soybean meals from test, control, and reference substances were evaluated in this study.

MAJOR COMPUTER SYSTEMS

The major computer systems used for this study included the following systems:

- Balance Application (balance weight capture system)
- ELAN (ICP-MS)
- Laboratory Information Management System (sample and assay tracking)
- Metasys or REES (monitor and document storage conditions for test/control/reference materials and samples, if applicable)
- eNotes (official study communication)
- Waters Empower®Chromatography Manager (data acquisition and result calculation system)
- ICP WinLab32 (ICP spectrometry)
- Labware Laboratory Information Management System (Reagent and solution preparation tracking

TEST, CONTROL AND REFERENCE SUBSTANCES

Test Substance
The test substance was soybean meal identified as SYHTOH2.

Control Substance
The control substance was soybean meal identified as Jack.

Reference Substance
The reference substance was soybean meal identified as Commercial.

Appropriate reference standards were used in each assay for the analytical procedures and equipment calibrations.

® Empower is a registered trademark of Waters Corporation

Storage Condition
Upon receipt, the samples were stored at ambient temperature. Reference standards were stored according to vendor specifications or as deemed appropriate.

Characterization, Purity, and Stability
The purpose of this study was to determine the composition of the samples. The sponsor has deemed that the samples were stable for the duration of the experimental phase of this study when stored as described in the protocol. Stability of the compositional analytes in the samples was not determined in this project.

Certificates of analysis of the reference standards will be archived at Covance. Reference standard stability [e.g., expiration, shelf life, retest date, re-certification date, or equivalent] was documented in the raw data.

Disposition
Any remaining prepared dilutions or extractions of the samples were discarded at Covance.

After the samples are analyzed, all excess samples will be retained at Covance Laboratories Inc. for one year in accordance with EPA 40 CFR Part 160 at which time the sponsor will be contacted for further disposition instructions.

Remaining reference standards may be used for other testing.

EXPERIMENTAL DESIGN

Sample Receipt and Handling
The samples were entered into the Covance Laboratory Information Management System (LIMS) with unique LIMS numbers. Each sponsor's sample identification was matched with the Covance LIMS information. Documentation of the samples upon receipt at Covance was maintained in the raw data. Samples were ground prior to being analyzed. Any sample processing was documented in the raw data.

Analytical Methods
This study used approved analytical methods to determine the composition of the samples.

The samples were analyzed singly unless otherwise dictated by Covance methods and/or SOPs. Quality control samples (duplicates, recoveries, certified reference standards, blanks, or validated control samples) were prepared and analyzed at Covance. Re-analyses were performed as determined by Covance methods and/or SOPs. When re-analyses were deemed necessary, documentation and justification were provided in the raw data.

The following analyses were performed on the **Soybean Meal** samples:

Analyte	Method Mnemonic[1]
Proximates	
Moisture	M100
Protein	PGEN
Fat	FSOX
Ash	ASHM
Mycotoxin Screen	
T2 Toxin	Romer Labs[2]
Deoxynivalenol (DON) and acetyl-DON	
Zearalenone	
Fumonisin (B1, B2, B3, total)	
Aflatoxins (B1, B2, G1, G2, total)	AHMF
Sulfur	MSS
Chloride	CL
Amino Acids	TALC
(threonine, cystine, valine, methionine, isoleucine, leucine, lysine, aspartic acid, glutamic acid, proline, glycine, alanine)	
Crude Fiber	CFIB
Minerals	ICPS
Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium, Zinc	
Starch	STCH

[1]Analytical methods are kept on file at Covance Laboratories Inc.
[2]These analyses were sub-contracted by Covance to Romer Labs. See Appendix A for the method summary.

Carbohydrate (CHO) values were determined by calculation.

CONTROL OF BIAS

The samples were treated identically during analysis to minimize assay bias.

STATISTICAL EVALUATIONS

There were no statistical evaluations performed on the final tabulated results by Covance. Any transformations, calculations, or operations performed were as described in the Covance methods.

RECORD RETENTION

Data relating to or generated by the project, including the original protocol, original protocol amendments and raw data study book will be forwarded to Colorado Quality Research upon finalizaton of the report except for the Romer Labs data, which will be maintained by Romer Labs according to their procedures. Copies of the protocol, protocol amendments, and final report will be kept in Covance Laboratories Inc. archives. Electronic data collected at Covance Laboratories Inc. using Empower software will be stored on duplicate compact discs (CDs). The CDs will be stored in the archives at Covance Laboratories Inc. A CD containing Empower data will be transferred to Colorado Quality Research upon completion of the report.

Supporting records that will be retained at Covance, but will not be archived with the study data, include:

- Instrument calibration and maintenance records
- Storage temperature records
- Training records of study personnel
- Durable media records
- Standard Operating Procedures
- Standard logbooks

RESULTS

The results for all analyses are presented in Table 1. All of the results are expressed on an “as received” basis.

PROTOCOL DEVIATION

The Experimental section of the protocol indicated the mycotoxin testing to be performed by Romer Labs. In addition to the mycotoxins listed in the protocol, Romer Labs also reported results for acetyl-deoxynivalenol (acetyl-DON). The results were below the limit of quantitation. There is no impact on the study. The results will be maintained with the study data.

Table 1
Nutritional Components and
Environmental Contaminants
of Soybean Meal

Material Name **Covance LIMS Number**	Commercial Control 11000744	SYHTOH2 Test 11000745	Jack Control 11000746
Proximate (%)			
Moisture	6.21	5.64	6.16
Protein	51.4	44.2	46.0
Total Fat	0.659	0.794	0.519
Ash	5.84	6.70	6.99
Carbohydrates	35.9	42.7	40.3
Crude Fiber (%)	4.41	3.43	3.01
Minerals (ppm)			
Copper	17.6	14.9	17.1
Iron	102	137	122
Manganese	26.8	30.2	31.1
Zinc	50.5	81.2	75.3
Minerals (%)			
Calcium	0.251	0.177	0.205
Magnesium	0.274	0.284	0.276
Phosphorus	0.714	1.08	1.03
Potassium	2.24	2.34	2.45
Sodium	< 0.0100	< 0.0100	< 0.0100
Chloride (%)	0.0356	0.0354	0.0349
Sulfur (%)	0.434	0.454	0.453
Starch (%)	0.892	3.67	2.03
Mycotoxins			
Aflatoxin B1 (ppb)	< 0.500	< 0.500	< 0.500
Aflatoxin B2 (ppb)	< 0.500	< 0.500	< 0.500
Aflatoxin G1 (ppb)	< 0.500	< 0.500	< 0.500
Aflatoxin G2 (ppb)	< 0.500	< 0.500	< 0.500
Total Aflatoxins (ppb)	< 2.00	< 2.00	< 2.00
T2 Toxin (ppm)	< 0.2	< 0.2	< 0.2
Deoxynivalenol (DON) (ppm)	< 0.6	< 0.6	< 0.6
Zearalenone (ppm)	< 51.7	< 51.7	< 51.7
Fumonisin B1 (ppm)	< 0.1	< 0.1	< 0.1
Fumonisin B2 (ppm)	< 0.1	< 0.1	< 0.1
Fumonisin B3 (ppm)	< 0.1	< 0.1	< 0.1
Total Fumonisins (ppm)	< 0.3	< 0.3	< 0.3

Table 1
Nutritional Components and
Environmental Contaminants
of Soybean Meal

Material Name	Commercial Control	SYHTOH2 Test	Jack Control
Covance LIMS Number	11000744	11000745	11000746
Amino Acids (%)			
Aspartic Acid	5.73	4.80	5.06
Threonine	1.95	1.75	1.83
Glutamic Acid	9.06	7.14	7.67
Proline	2.62	2.14	2.26
Glycine	2.12	1.81	1.92
Alanine	2.21	1.88	2.00
Cystine	0.701	0.710	0.696
Valine	2.39	2.09	2.19
Methionine	0.666	0.605	0.620
Isoleucine	2.39	2.02	2.11
Leucine	3.74	3.18	3.36
Lysine	3.13	2.70	2.79

APPENDIX A
Analytical Method Summaries and Reference Standards

Analytical Method Summaries and Reference Standards

Aflatoxins (AHMF)
Aflatoxins were extracted from samples with a polar solvent/water solution using a high-speed blender. Analysis was performed with high-performance liquid chromatography equipped with post-column derivatization and fluorescence detection. The results are reported on an "as received" basis. The limit of quantitation was 0.500 ppb.

Reference Standards:
Biopure, Aflatoxin B1, 99% ±1%, Lot No. L10203A
Biopure, Aflatoxin B2, 99% ±1%, Lot No. L10271B and LR10521B
Biopure, Aflatoxin G1, 99% ±1%, Lot No. LR09513C
Biopure, Aflatoxin G2, 99% ±1%, Lot Nos. L10521D and LR10203C

References:
Official Methods of Analysis of AOAC INTERNATIONAL, Methods 991.31 and 999.07, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD (2011).

Amino Acid Composition (TALC)
Total aspartic acid (including asparagine)
Total threonine
Total glutamic acid (including glutamine)
Total proline
Total glycine
Total alanine
Total valine
Total isoleucine
Total leucine
Total lysine
Total methionine
Total cystine (including cysteine)

The samples were hydrolyzed in 6N hydrochloric acid for approximately 24 hours at approximately 106-118ºC. Phenol was added to the 6N hydrochloric acid to prevent halogenation of tyrosine. Cystine and cysteine are converted to S-2-carboxyethyl-thiocysteine by the addition of dithiodipropionic acid.

The samples were analyzed by HPLC after pre-injection derivatization. The primary amino acids were derivatized with o-phthalaldehyde (OPA) and the secondary amino acids are derivatized with fluorenylmethyl chloroformate (FMOC) before injection. The results are reported on an "as received basis". The limit of quantitation for this study was calculated as 0.0100%.

Reference Standards:

Component	**Manufacturer**	**Lot No.**	**Purity** (%)
L-Alanine	Sigma-Aldrich	1440397	99.9
L-Aspartic Acid	Sigma-Aldrich	BCBB9274	100.6
L-Cystine	Sigma-Aldrich	1418036	99.9
L-Glutamic Acid	Sigma-Aldrich	1423805	100.2
Glycine	Sigma-Aldrich	1119375	100
L-Isoleucine	Sigma-Aldrich	1423806	100
L-Leucine	Sigma-Aldrich	BCBB1733	98.6
L-Lysine Monohydrochloride	Sigma-Aldrich	1362380	100.2
L-Methionine	Sigma-Aldrich	1423807	99.9
L-Proline	Sigma-Aldrich	1414414	99.7
L-Threonine	Sigma-Aldrich	1402329	100
L-Valine	Sigma-Aldrich	1352709	100

References:

R. Schuster "Determination of Amino Acids in Biological, Pharmaceutical, Plant and Food Samples by Automated Precolumn Derivatization and High-Performance Liquid Chromatography", J. Chromatogr., 431, 271-284 (1988).

Henderson, J. W., Ricker, R. D., Bidlingmeyer, B. A., Woodward, C., "Rapid, Accurate, Sensitive, and Reproducible High-Performance Liquid Chromatography Analysis of Amino Acids, Amino Acid Analysis Using Zorbax Eclipse-AAA columns and the Agilent 1100 High-Performance Liquid Chromatography," Agilent Publication 5980-1193 (2000).

Henderson, J. W., Brooks, A., Agilent Application Note 5990-4547, "Improved Amino Acid Methods using Agilent Zorbax Eclipse Plus C18 Columns for a Variety of Agilent LC Instrumentation and Separation Goals" (2010).

Barkholt and Jensen, “Amino Acid Analysis: Determination of Cysteine plus Half-Cystine in Proteins after Hydrochloric Acid Hydrolysis with a Disulfide Compound as Additive,” Analytical Biochemistry, 177, 318-322 (1989).

Ash (ASHM)

The sample was placed in an electric furnace at 550°C and ignited. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The results are reported on an “as received” basis. The limit of quantitation was 0.100%.

Reference:

Official Methods of Analysis of AOAC INTERNATIONAL, Method 923.03, AOAC INTERNATIONAL: Gaithersburg, MD (2011).

Carbohydrate (CHO)
The total carbohydrate level was calculated by difference using the "as received" weight-derived data and the following equation:

% carbohydrates = 100 % - (% protein + % fat + % moisture + % ash)

The limit of quantitation was calculated as 0.100% on an "as received" basis.

Reference:
United States Department of Agriculture, "Energy Value of Foods", *Agriculture Handbook No. 74,* pp. 2-11, (1973).

Chloride (CL)
The samples were put into solution with double-deionized water and then made acidic with nitric acid. Chloride was determined potentiometrically by titrating with a standard silver nitrate solution to a predetermined endpoint. The results are reported on an "as received" basis. The limit of quantitation was 0.00400%.

References:
Official Methods of Analysis of AOAC INTERNATIONAL, Methods 963.05, 971.27, and 986.26, AOAC INTERNATIONAL, Gaithersburg, MD (2011).

Crude Fiber (CFIB)
Crude fiber was quantitated as the loss on ignition of dried residue remaining after digestion of the sample with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions. The results are reported on an "as received" basis. The limit of quantitation was 0.100%.

References:
Official Methods of Analysis of AOAC INTERNATIONAL, Method 962.09, AOAC INTERNATIONAL, Gaithersburg, MD (2011).

Official Methods and Practices of the American Oil Chemists' Society 5th Edition, AOCS Method Ba 6a-05, American Oil Chemists' Society, IL (2006).

Fat by Soxhlet Extraction (FSOX)
The sample was weighed into a cellulose thimble containing sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed. The results are reported on an "as received" basis. The limit of quantitation for this study was 0.100%.

References:
Official Methods of Analysis of AOAC INTERNATIONAL, Methods 960.39 and 948.22, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD (2011).

ICP-Mass Spectrometry (MSS)
The sample was wet-ashed with nitric acid using microwave digestion. Using inductively coupled plasma mass spectrometry, the amount of each element was determined by comparing the counts generated by the unknowns to those generated by standard solutions of known concentrations. The results are reported on an "as received" basis. The following lists the limit of quantitation.

Spex CertiPrep Reference Standards and Limits of Quantitation:

Mineral	**Lot No.**	**Concentration** (mg/L)	**Limit of Quantitation**
Sulfur	16-151S	1000	0.00100%

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL, Method 993.14, AOAC INTERNATIONAL, Gaithersburg, MD (2011).

ICP Emission Spectrometry (ICPS)
The sample was dried, precharred, and ashed overnight in a muffle set to maintain 500°C. The ashed sample was re-ashed with nitric acid, treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured on the inductively coupled plasma spectrometer, with the emission of the standard solutions. The results are reported on an "as received" basis. The limits of quantitation are listed below:

Inorganic Ventures Reference Standards and Limits of Quantitation:

Mineral	**Lot No.**	**Concentration** (µg/mL)	**LOQ**
Calcium	E2-MEB393070MCA, E2-MEB393072	200, 1000	0.00200%
Copper	E2-MEB393070MCA, E2-MEB393071MCA	2.00, 10.0	0.500 ppm
Iron	E2-MEB393070MCA, E2-MEB393073	10.0, 50.0	2.00 ppm
Magnesium	E2-MEB393070MCA, E2-MEB393071MCA	50.0, 250	0.00200%

Mineral	Lot No.	Concentration (µg/mL)	LOQ
Manganese	E2-MEB393070MCA, E2-MEB393071MCA	2.00, 10.0	0.300 ppm
Phosphorus	E2-MEB393070MCA, E2-MEB393072	200, 1000	0.00200%
Potassium	E2-MEB393070MCA, E2-MEB393072	200, 1000	0.0100%
Sodium	E2-MEB393070MCA, E2-MEB393072	200, 1000	0.0100%
Zinc	E2-MEB393070MCA, E2-MEB393071MCA	10.0, 50.0	0.400 ppm

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL, Methods 984.27 and 985.01, AOAC INTERNATIONAL, Gaithersburg, MD (2011).

Moisture (M100)
The sample was dried in a vacuum oven at approximately 100°C to a constant weight. The moisture weight loss was determined and converted to percent moisture. The results are reported on an "as received". The limit of quantitation was 0.100%.

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL Methods 926.08 and 925.09, AOAC INTERNATIONAL, Gaithersburg, MD (2011).

Protein (PGEN)
The protein and other organic nitrogen in the sample were converted to ammonia by digesting the sample with sulfuric acid containing a catalyst mixture. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25. The results are reported on an "as received" basis. The limit of quantitation was 0.100%.

References:
Official Methods of Analysis of AOAC INTERNATIONAL, Methods 955.04 and 979.09, AOAC INTERNATIONAL, Gaithersburg, MD (2011)

Official Methods and Recommended Practices of the AOCS, 5th Edition, Method Ac 4-91, American Oil Chemists' Society, Champaign, IL (1997).

Starch (STCH)
The sample was extracted with alcohol to remove carbohydrates other than starch, i.e. sugars. Then it was hydrolyzed into glucose with α-amylase and amyloglucosidase. Glucose was oxidized with glucose oxidase to form peroxide, which reacted with a dye in the presence of peroxidase to give a stable colored product proportional to glucose

concentration. The glucose concentration was quantitated by measurement on a spectrophotometer at 510 nm. Percent starch was then calculated from the glucose concentration. The results are reported on an "as received" basis. The limit of quantitation was 0.0500%.

Reference Standard:
Sigma-Aldrich D-(+)-Glucose, 99.8% Lot No. 080M0142V

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL, Method 996.11, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD (2011).

Mycotoxins by LC-MS/MS (Romer Labs Inc.)
The sample was extracted with acetonitrile:water, filtered, dried down and reconstituted. The extract was analyzed on an LC-MS/MS system, using positive mode for fumonisins and T2 toxin and negative mode for deoxynivalenole and zearalenone. The limits of quantitation were calculated and reported on an "as received" basis. The limits of quantitation for this study were:

T2 Toxin:	<0.2 ppm
Deoxynivalenol (DON):	<0.6 ppm
Acetyl-DON*:	<0.8 ppm
Zearalenone	<51.7 ppm
Fumonisin B1	<0.1 ppm
Fumonisin B2	<0.1 ppm
Fumonisin B3	<0.1 ppm

*See Protocol Deviation, p. 12.

References:
Romer 000045-1-LWI-Multitoxin Mycotoxin Detection Method by LC-MSMS

Sulyok et al., "Development and Validation of a Liquid Chromatography/Tandem Mass Spectrometric Method for the Determination of 39 Mycotoxins in Wheat and Maize.", Rapid Communications in Mass Spectrometry, Vol. 20(18), pp 2649-2659 (2006).

Binder, E.M., "Romer Spotlight", Vol. 8.

Statistical Analysis Report

The Effect of Diets Containing Soybean Meal from SYHT0H2, Nontransgenic Control and a Commercial Variety on Broiler Growth Performance and Carcass Parameters

Study No. SYN-11-1 (Syngenta Study TK0036575)

Sponsor:

Syngenta Biotechnology, LLC
3054 East Cornwallis Road
PO Box 12257
Research Triangle Park, NC 27709-2257, USA

Study location:

Colorado Quality Research, Inc. (CQR)
400 East County Road 72
Wellington, CO 80549

Prepared By:

[redacted]

Biostatistician
890 Bren Del Drive
Petoskey, MI 49770

[redacted]

July 24, 2012
Date

REGULATORY COMPLIANCE STATEMENT

The Statistical Report for Syngenta Biotechnology, LLC, titled **" The Effect of Diets Containing Soybean Meal from SYHT0H2, Nontransgenic Control and a Commercial Variety on Broiler Growth Performance and Carcass Parameters"** was conducted in accordance with:

**Good Laboratory Practices for Non-Clinical Laboratory Studies,
U.S. Code of Federal Regulations (CFR), Title 21, Chapter 1, Part 58.**

Furthermore, all software used was validated as per GLP regulations.

This Statistical Report is a true and accurate record of the procedures and results obtained.

[redacted]
Statistician

July 24, 2012
Date

Objective:

To evaluate the nutritional value of diets containing soybean meal produced from SYHT0H2, nontransgenic, near-isogenic control and commercial varieties.

Study Design:

Treatments were assigned to pens using a randomized complete block design. The test facility was divided into 5 blocks of 6 pens each. Birds were assigned to the pens randomly within gender according to CQR SOP B-10. The general study design was as follows:

Treatment [1]	Soybean meal ID	No. of Pens of Each Sex	No. of Males /Pen	No. of Females /Pen	Total No. of Birds/Sex	Total No. Birds/ Treatment
1	SYHT0H2 Test	5	12	12	60	120
2	Nontransgenic Control	5	12	12	60	120
3	Commercial Variety	5	12	12	60	120
	Total	30			180	360

[1] Treatment identity remained blinded until the in-life phase of the study was completed.

Performance data were calculated and summarized by average weight gain per bird on Day 42. The average feed:gain was calculated for Days 0 - 42 by dividing the total feed consumption by the total weight gain of surviving birds for that pen. Adjusted feed:gain was calculated by dividing the total feed consumption by the weight gain of surviving birds plus weight gain of birds that died or were removed from that pen.

The following yield data for individual birds was collected:

- Carcass weight
- Breast meat weight –skinless, boneless
- Wings (bone in, skin on)
- Thighs (bone in, skin on)
- Drums (bone in, skin on)

Calculations were conducted to express parts on a percentage basis of carcass weight. This was done by dividing the weight of the part by the weight of the carcass from which it came and multiplying by 100.

Statistical Methods:

Statistical analyses were conducted on growth performance (body weight), carcass weight and parts, feed consumption, and feed conversion ratio (unadjusted for mortality). The pen was used as the experimental unit. Statistical significance was defined as alpha = 0.05. ANOVA was used to assess treatment related differences. The statistical model included block, all treatments, gender and the treatment by gender interaction as fixed effects. If the gender by treatment

interaction was not significant, the main effect of treatment was evaluated. If the gender by treatment interaction was significant ($P < 0.05$), this may be judged to undermine the validity of comparing treatments across genders, in which case within gender treatment effects were assessed.

Within the model framework stated above, the significance of the comparisons between treatments was also determined in all cases, regardless of the significance of the overall treatment effect (two-sided test).

Results:

The summary of the ANOVA for the performance outcomes is provided in Tables 1 and 2. No statistically significant differences associated with treatment group were observed ($P > 0.05$, Table 1). Additionally, pairwise comparisons of treatment groups revealed no statistically significant differences (Table 2). There were no significant differences between the SYHT0H2 Test and the non-transgenic control groups.

The summary of the ANOVA for the carcass outcomes is provided in Tables 3, 4 and 5. The treatment by sex interaction was significant for thigh weight (Table 3). Evaluation of the treatment effect within gender indicated no significant differences between the SYHT0H2 Test and the non-transgenic control groups (Table 4). No treatment effect was detected for females (Table 4) with regard to thigh weights. No statistically significant differences associated with treatment group were observed for the remaining carcass measurements ($P > 0.05$, Table 3). Pairwise comparisons of treatment groups indicated there were no significant differences between the SYHT0H2 Test and the non-transgenic control groups (Table 5).

Table 1: Summary of the ANOVA for performance outcomes - P-values

Variable	P-values			
	Block	**Treatment**	**Sex**	**Treatment x Sex interaction**
Day 42 body weight (pen basis)	0.6379	0.2079	0.0009	0.3374
Gain per bird (day 0 - 42)	0.0758	0.6246	<.0001	0.5736
Gain per pen (day 0 - 42)	0.6337	0.2095	0.0009	0.3392
Feed Intake (pen basis)	0.5621	0.4113	0.0010	0.5607
Feed to gain ratio (unadjusted for mortality)	0.3837	0.1247	0.0121	0.2451
Feed to gain ratio (adjusted for mortality)	0.2821	0.4599	<.0001	0.2234
Gain per pen (adjusted for mortality)	0.6639	0.3535	<.0001	0.4757

Table 2: Least squares means for performance outcomes

Variable	Treatment 1	Treatment 2	Treatment 3	SEM
Day 42 body weight, kg (pen basis)	31.02	33.17	33.11	0.9401
Gain per bird, kg (day 0 - 42)	2.89	2.92	2.92	0.0230
Gain per pen, kg (day 0 - 42)	30.53	32.68	32.63	0.9409
Feed Intake, kg (pen basis)	50.77	52.29	51.70	0.7978
Feed to gain ratio (unadjusted for mortality)	1.68	1.61	1.59	0.0297
Feed to gain ratio (adjusted for mortality)	1.53	1.54	1.53	0.0054
Gain per pen, kg (adjusted for mortality)	33.15	34.11	33.92	0.4845

Table 3: Summary of the ANOVA for carcass outcomes - P-values†

Variable	P-values			
	Block	**Treatment**	**Sex**	**Treatment x Sex interaction**
Breast Weight	0.3102	0.2236	<.0001	0.7802
Breast percent	0.1992	**0.0438**	<.0001	0.7027
Drum Weight	0.0062	0.2387	<.0001	0.5480
Drum percent	0.0399	0.0641	<.0001	0.9402
Live Weight	0.1370	0.8219	<.0001	0.2762
Thigh Weight	0.3092	0.5357	<.0001	**0.0466**
Thigh percent	0.8829	0.2468	0.0109	0.1884
Wing Weight	0.2503	0.9121	<.0001	0.1394
Wing percent	0.7242	0.2199	0.6571	0.3036
Hot percent	0.7355	0.2453	0.0930	0.1661
Hot Weight	0.2163	0.5364	<.0001	0.5938

†P-values bolded are significantly different at P<0.05 or equivalent.

Table 4: Least squares means for carcass outcomes - sex * treatment

Variable	Sex	Treatment 1	Treatment 2	Treatment 3	SEM
Thigh Weight	F	0.31	0.31	0.31	0.004
	M	0.36a	0.37ab	0.38b	0.004

a,b: values with no common letter are significantly different at $P < 0.05$

Table 5: Least squares means for carcass outcomes

Variable	Treatment 1	Treatment 2	Treatment 3	SEM
Breast percent	32.52%ab	32.91%a	32.15%b	0.20%
Breast weight	0.66	0.68	0.66	0.009
Drum percent	12.48%ab	12.45%a	12.68%b	0.07%
Drum weight	0.26	0.26	0.26	0.002
Live weight	2.83	2.84	2.86	0.030
Thigh weight	0.34	0.34	0.34	0.003
Thigh percent	16.55%	16.31%	16.62%	0.13%
Wing percent	10.13%	9.94%	10.01%	0.08%
Wing weight	0.21	0.21	0.21	0.002
Hot percent	71.94%	72.94%	71.96%	0.47%
Hot weight	2.04	2.07	2.06	0.019

a,b: values with no common letter are significantly different at $P < 0.05$

APPENDIX F Syngenta Analytical Phase

syngenta

The Effect of Diets Containing Soybean Meal from SYHT0H2, Nontransgenic Control and a Commercial Variety on Broiler Growth Performance and Carcass Parameters

Analytical Phase Report

DATA REQUIREMENT(S): Not Applicable

AUTHOR(S): [redacted]

ANALYTICAL PHASE COMPLETION DATE: June 11, 2012

PERFORMING LABORATORY:
Syngenta Crop Protection, LLC
Product Safety
3054 East Cornwallis Road
Research Triangle Park, NC 27709-2257 USA

LABORATORY PROJECT ID:
Report Number: SYN-11-1
Task Number: TK0036575

SPONSOR:
Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

PAGE 1 OF 19

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This phase of the study was conducted in compliance with the relevant provisions of Good Laboratory Practice Standards (GLPS) (40 CFR Part 160, US EPA 1989) pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) with the following exception:

- Real-time polymerase chain reaction copy control substances were not characterized in accordance with EPA FIFRA GLPS.

Principal Investigator:

[redacted] June 12, 2012

Date

Technical Expert, Product Safety
Syngenta Crop Protection, LLC

QUALITY ASSURANCE STATEMENT

Study Title: The Effect of Diets Containing Soybean Meal from SYHT0H2, Nontransgenic Control and a Commercial Variety on Broiler Growth Performance and Carcass Parameters

Study Director: [redacted]

Analytical Principal Investigator: [redacted]

Study Number: SYN-11-1

Syngenta Task Number: TK0036575

Pursuant to Good Laboratory Practice Regulations (40 CFR Part 160), this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

Inspection/Audit Type	Inspection/Audit Dates	Reporting Date
Inspect Analytical	March 1, 2012	March 2, 2012
Audit Phase Report (1st Audit)	April 19 – 23, 2012	April 24, 2012
Audit Phase Report (2nd Audit)	May 22, 2012	May 23, 2012

Prepared by [redacted] Date: June 11, 2012

Staff Quality Assurance Auditor
Quality Assurance Unit
Syngenta

GENERAL INFORMATION

Contributors

The following contributed to this analytical phase report in the capacities indicated:

Name	Title
[redacted]	Principal Investigator, Syngenta Crop Protection, LLC
[redacted]	Sample Analyst, Syngenta Crop Protection, LLC
[redacted]	Sample Analyst, Syngenta Crop Protection, LLC
[redacted]	Sample Analyst, Syngenta Biotechnology, Inc.
[redacted]	Sample Preparation, Syngenta Crop Protection, LLC

Study Dates

Analytical phase start date: February 08, 2012
Analytical phase termination date: April 11, 2012

Records Retention

Raw data, the original copy of this report, and other relevant records are archived at Syngenta, 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257 USA. Verified copies of the raw data, phase report, and other relevant records will be provided to the study director.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ACRONYMS AND ABBREVIATIONS

avhppd-03	*p*-hydroxyphenylpyruvate dioxygenase gene derived from *Avena sativa* (oat)
AvHPPD-03	*p*-hydroxyphenylpyruvate dioxygenase enzyme encoded by *avhppd-03*
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	gram
GLPS	Good Laboratory Practice Standards
HPPD	*p*-hydroxyphenylpyruvate dioxygenase enzyme
LOD	limit of detection
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
US EPA	United States Environmental Protection Agency
w/w	weight to weight
µg	microgram

1.0 EXECUTIVE SUMMARY

The purpose of this analytical phase of this study was to determine the concentrations of the proteins *p*-hydroxyphenylpyruvate dioxygenase (AvHPPD-03) and phosphinothricin acetyltransferase (PAT) in meal processed from the seed of soybean derived from transformation Event SYHT0H2 and in broiler chicken diets prepared with SYHT0H2 soybean meal. The broiler chicken diets were used in a broiler chicken feeding study.

Soybean (*Glycine max* [L.] Merrill) has been genetically modified to express the novel genes *avhppd-03* derived from oat (*Avena sativa* L.) and *pat* from *Streptomyces viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in transgenic Event SYHT0H2 soybean plants confers a mesotrione-tolerance phenotype. The gene *pat* encodes the enzyme phosphinothricin acetyltransferase. Expression of *pat* confers a glufosinate-tolerance phenotype, which was used as a selectable marker in the development of SYHT0H2 soybean.

Enzyme-linked immunosorbent assays were used to quantify AvHPPD-03 and PAT in samples of SYHT0H2 soybean meal and meal prepared from a nontransgenic, near-isogenic soybean. The AvHPPD-03 and PAT proteins were below the limit of detection in both the SYHT0H2 soybean meal and nontransgenic, near-isogenic soybean meal. However, Event-specific PCR testing confirmed the presence of SYHT0H2 DNA in the SYHT0H2 soybean meal and corresponding diets. Event-specific PCR testing also confirmed the absence of SYHT0H2 DNA in the nontransgenic control soybean meal, the commercial soybean meal, and each of the corresponding broiler chicken diets.

2.0 INTRODUCTION

The purpose of this analytical phase of this study was to measure the concentrations of the proteins *p*-hydroxyphenylpyruvate dioxygenase (AvHPPD-03) and phosphinothricin acetyltransferase (PAT) in meal processed from the seed of soybean derived from transformation Event SYHT0H2 and in broiler chicken diets prepared with that meal. The broiler-chicken diets were used in a feeding study.

Soybean (*Glycine max* [L.] Merrill) has been genetically modified to express the novel genes *avhppd-03* derived from oat (*Avena sativa* L.) and *pat* from *Streptomyces viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03, that catalyzes the formation of homogentisic acid, the aromatic precursor in plastoquinone and vitamin E biosynthesis. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in transgenic Event SYHT0H2 soybean plants confers a mesotrione-tolerance phenotype. The gene *pat* encodes the enzyme phosphinothricin acetyltransferase, which inactivates the herbicide glufosinate, an inhibitor of glutamine synthetase, an enzyme in the nitrogen assimilation pathway. Expression of *pat* confers a glufosinate-tolerance phenotype, which was used as a selectable marker in the development of SYHT0H2 soybean.

Enzyme-linked immunosorbent assays (ELISA) were used to measure AvHPPD-03 and PAT concentrations in the soybean meal samples, and real-time polymerase chain reaction (PCR) testing was used to confirm the genetic identity of the soybean meal and corresponding diets.

3.0 MATERIALS AND METHODS

3.1 Test, Control, and Commercial Material, and Reference Substances

The test material for this study was soybean meal derived from SYHT0H2 soybean seed in the genetic background 'Jack' (Nickell *et al.* 1990). The control material was soybean meal derived from nontransgenic, near-isogenic soybean seed of the same genetic background as the test substance. Table 1 shows the descriptions and material identification codes for the test, control, and commercial material seed.

TABLE 1 Test, control, and commercial materials

Seed identification	Material identification
Nontransgenic (control)	10RS000015
SYHT0H2 (test)	10RS000001
Commercial variety (Commercial material)	S03-18-20-23

Table 2 shows the protein reference substances used to produce the standard curve for each ELISA.

TABLE 2 Protein reference substances for ELISA analyses

Protein	Reference substance ID	Characterization report
AvHPPD-03	AvHPPD-03-0209	Winslow 2009
PAT	PAT-0109	Seastrum 2009

3.2 Broiler Chicken Diets

Defatted toasted meal was prepared from the seed of SYHT0H2 soybean and a nontransgenic, near-isogenic control soybean by the Food Protein Research and Development Center, Texas A&M University, Bryan TX, USA. Laboratory-scale methodology equivalent to industry-standard processing was used.

The following broiler chicken diets were prepared with the defatted toasted meal at Colorado Quality Research, Inc., Wellington, CO, USA:

Starter diet containing 33.5% (w/w) SYHT0H2 soybean meal
Starter diet containing 33.5% (w/w) nontransgenic control soybean meal
Starter diet containing 32.9% (w/w) commercial soybean meal
Grower/Finisher diet containing 30% (w/w) SYHT0H2 soybean meal
Grower/Finisher diet containing 30% (w/w) nontransgenic control soybean meal
Grower/Finisher diet containing 29% (w/w) commercial soybean meal

Samples of the soybean meal and the diets were transported on dry ice to Syngenta Crop Protection, LLC, Research Triangle Park NC, USA, and stored at -80ºC ± 10ºC upon receipt.

3.3 Sample Preparation

Soybean meal and diet samples were ground to a fine powder in the presence of dry ice. Nontransgenic samples were processed first to prevent possible cross-contamination. Each powdered sample was mixed thoroughly to ensure homogeneity and stored at -80ºC ± 10ºC.

3.4 Protein Extraction and ELISA Analyses

Protein extractions were performed on three representative aliquots of the SYHT0H2 soybean meal sample and one representative aliquot of the nontransgenic control soybean meal sample. ELISA methodology was used to quantify AvHPPD-03 and PAT in each extract. For each ELISA, a standard curve was generated with known amounts of the corresponding reference protein. The mean absorbance for each sample extract was plotted against the appropriate standard curve to obtain the amount of AvHPPD-03 and PAT as nanograms per milliliter of extract.

A description of the AvHPPD-03 and PAT quantification procedures, including validation of ELISA sensitivity and extraction efficiency, can be found in Appendices A and B.

3.5 Event-Specific PCR Analysis

Qualitative event-specific PCR was used to confirm the presence of the Event SYHT0H2 deoxyribonucleic acid (DNA) in the SYHT0H2 soybean meal and corresponding diet samples and to confirm its absence in the nontransgenic control soybean meal, the commercial soybean meal, and corresponding diet samples. A description of the event-specific PCR analysis can be found in Appendix C.

3.6 Control of Bias Statement

Representative aliquots were analyzed from homogeneous samples. For both ELISA and event-specific real-time PCR testing, extracts from the test, control, and commercial samples were analyzed concurrently. Any rejected data, and the documented reasons for the rejection of those data, are retained in the study file.

3.7 Statistical Analysis Statement

All calculations were performed with Microsoft Excel® 2007 spreadsheet software.

4.0 RESULTS

The proteins AvHPPD-03 and PAT were not detected in the SYHT0H2 soybean meal or the nontransgenic control soybean meal. The limits of detection (LOD) were 0.063 µg/g for AvHPPD-03 and 0.05 µg/g for PAT in soybean meal (limits can be found in Appendices A and B). Because the proteins were not detected in the SYHT0H2 soybean meal, the diets were not analyzed by ELISA.

Qualitative event-specific real-time PCR analysis confirmed the presence of Event SYHT0H2 DNA in the test soybean meal and the corresponding diets, confirming that the test diets contained soybean meal prepared from SYHT0H2 soybean. Event-specific PCR analysis also confirmed the absence of Event SYHT0H2 DNA in the nontransgenic control soybean meal, the commercial soybean meal, and each of the corresponding broiler chicken diets.

4.1 Data Quality and Integrity

No circumstances occurred during the conduct of this study that would have adversely affected the quality or integrity of the data generated.

5.0 REFERENCES

Nickell CD, Noel GR, Thomas DJ, Waller R. 1990. Registration of 'Jack' soybean. *Crop Sci* 30:1365.

Seastrum L. 2009. *Characterization of Microbially Produced Test Substance Containing Phosphinothricin Acetyltransferase (PAT) and Certificate of Analysis*. Report No. SSB-042-09 (unpublished). Research Triangle Park, NC: Syngenta Biotechnology, Inc.

US EPA. 1989. *Good Laboratory Practices Standards*. 40 CFR Part 160.

Winslow S. 2009. *Characterization of Microbially Produced Test Substance Containing* p-*Hydroxyphenylpyruvate Dioxygenase Protein (AvHPPD-03) and Certificate of Analysis*. Report No. SSB-041-09 (unpublished). Research Triangle Park, NC: Syngenta Biotechnology, Inc.

APPENDICES SECTION

APPENDIX A AvHPPD-03 Quantification Procedure

Reagents and buffers used for extraction and enzyme-linked immunosorbent assay (ELISA) analysis of AvHPPD-03

Buffer / Item	Constituents
Phosphate-buffered saline with 0.05% Tween 20 (PBST)	138 mM sodium chloride, 2.7 mM potassium chloride, 10.1 mM disodium phosphate, 1.8 mM potassium dihydrogen phosphate, pH 7.4, 0.05% Tween 20
Qualiplate™ Kits for HPPD in Soy	96-well plate precoated with anti-AvHPPD-03 antibody, AvHPPD-03 enzyme conjugate, substrate solution

AvHPPD-03 Extraction

PBST buffer was added to samples at a ratio of 3 ml of buffer to 15 mg of sample. The samples were mixed, homogenized in an Omni-Prep Multi-Sample Homogenizer, placed on wet ice to incubate for at least an hour, and centrifuged at 2°C to 8°C to form a pellet. The supernatants were removed and either analyzed the same day or stored at 2°C to 8°C prior to analysis.

AvHPPD-03 Quantification

The appropriate number of 96-well plates pre-coated with the capture antibody, the appropriate amounts of antibody/enzyme conjugate, and substrate solution were removed from storage at 2°C to 8°C and allowed to equilibrate to room temperature (all aforementioned items are provided in the Qualiplate™ ELISA Kit for HPPD in Soy). The tube containing the substrate solution was covered to prevent exposure to light. Dilutions of each sample extract and the ELISA standard (prepared using protein reference substance AvHPPD-03-0209 [Winslow 2009]), prepared in PBST, were applied to the plates at a volume of 50 µl/well. The plates were incubated at room temperature for at least 30 minutes while shaking. The plates were then washed five times prior to addition of the AvHPPD-03 enzyme conjugate (50 µl/well) and incubated at room temperature for 30 minutes while shaking. The plates were then washed five times prior to addition of the substrate solution (100 µl/well). The plates were covered to prevent exposure to light during incubation at room temperature for 5 to 15 minutes while shaking. The colorimetric reaction was stopped by the addition of 1 N hydrochloric acid (100 µl/well) and absorbance was measured using a spectrophotometer at 450 nm and 650 nm. The results were analyzed with Molecular Devices SoftMax Pro® GxP Microplate Data Compliance Software, v. 5.4.1. The 650-nm reference measurement was subtracted from the 450-nm measurement prior to further analysis. The sample results were interpolated from a standard curve generated through the use of a four-parameter algorithm.

Validation of AvHPPD-03 ELISA Extraction Efficiency and Sensitivity

Method sensitivity (minimum dilution factor, limit of detection [LOD], and limit of quantitation [LOQ]) for soybean meal was determined and is summarized below. Extraction efficiency for soybean meal was not determined as AvHPPD-03 was not detected.

Minimum dilution factor. The minimum dilution factor for each sample type was determined by analysis of a dilution series of nontransgenic extracts spiked with a known quantity of AvHPPD-03 reference protein. The most concentrated dilution of spiked sample extract that yielded a percent recovery between 70% and 120% and was followed by two subsequent dilutions with recoveries in the same range was selected as the minimum acceptable dilution factor.

Limit of detection. The LOD for each sample type was evaluated by comparison of the mean optical density (OD) plus three standard deviations of the unspiked nontransgenic sample extract with the mean OD of the nontransgenic sample extract spiked with AvHPPD–03 reference protein. The measured LOD is the lowest spike concentration with an OD greater than the mean OD plus three standard deviations of the unspiked nontransgenic sample extract.

The LOD (micrograms per gram of sample) was calculated by the following formula:

$$\left(\frac{\text{LOD (ng/ml)} \times \text{dilution factor} \times \text{volume of extraction buffer (ml)}}{\text{amount of tissue extracted (g)}}\right) \div 1000$$

Limit of quantitation. The LOQ for each sample type was evaluated by spiking of nontransgenic sample extracts with known concentrations of AvHPPD-03 reference protein and measurement of the percent recovery of AvHPPD-03 protein. The LOQ was the lowest spike concentration of AvHPPD-03 that resulted in recovery of between 70% and 120% of nominal value and was greater than or equal to the LOD.

The percent recovery for each spiked sample was calculated by the following formula:

$$\left(\frac{\text{mean protein concentration of spiked extract (ng/ml)}}{\text{spiked protein concentration (ng/ml)}}\right) \times 100$$

The LOQ (micrograms per gram of tissue) was calculated by the following formula:

$$\left(\frac{\text{LOQ (ng/ml)} \times \text{dilution factor} \times \text{volume of extraction buffer (ml)}}{\text{amount of tissue extracted (g)}}\right) \div 1000$$

Extraction efficiency. Extraction efficiency from soybean meal was not determined as AvHPPD-03 was not detected.

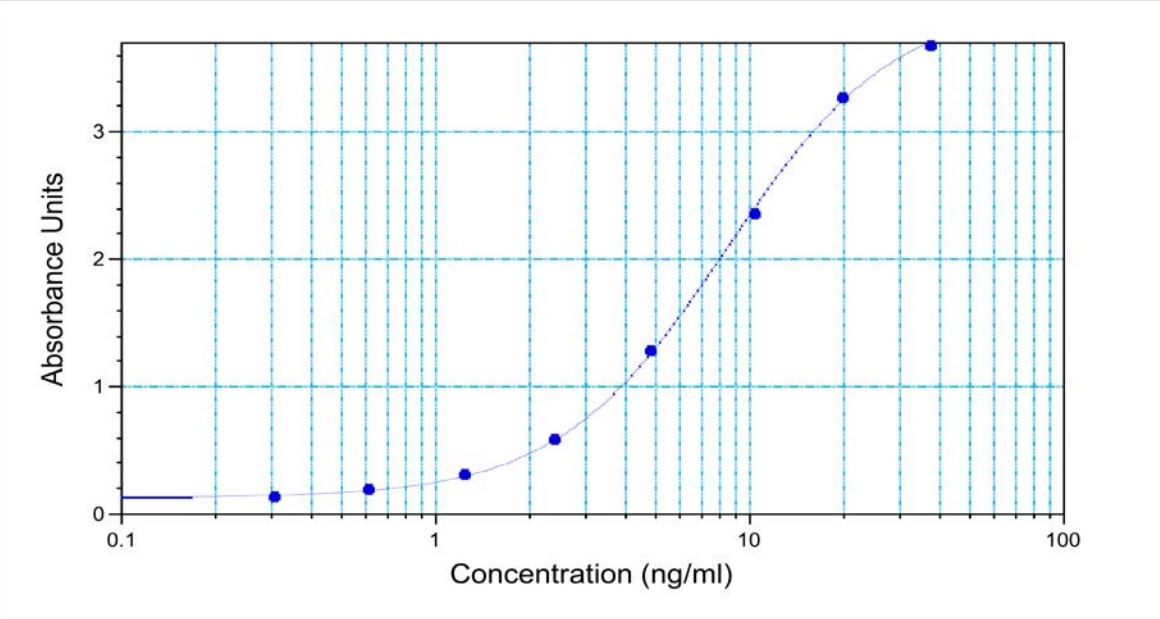
Method sensitivity data are summarized in the following table.

Minimum dilution factor, LOD, LOQ and extraction efficiency for each matrix

Sample Type	Minimum Dilution Factor	LOD (µg/g) FW	LOQ (µg/g) FW	Extraction Efficiency (%)
Soybean meal	1	0.063	0.125	–[a]

[a] Not determined because amount measured was less than LOD.

Representative Standard Curve. The concentrations used to produce the ELISA standard curve were 40, 20, 10, 5, 2.5, 1.25, 0.625, and 0.313 ng/ml. A representative standard curve for the AvHPPD-03 ELISA is shown below.



References

Winslow S. 2009. *Characterization of Microbially Produced Test Substance Containing* p-*Hydroxyphenylpyruvate Dioxygenase Protein (AvHPPD-03) and Certificate of Analysis.* Report No. SSB-041-09 (unpublished). Research Triangle Park, NC: Syngenta Biotechnology, Inc.

APPENDIX B PAT Quantification Procedure

Reagents and buffers used for extraction and enzyme-linked immunosorbent assay (ELISA) analysis of PAT

Buffer / Item	Constituents
Phosphate-buffered saline with 0.05% Tween® 20 (PBST)	138 mM sodium chloride, 2.7 mM potassium chloride, 10.1 mM disodium phosphate, 1.8 mM potassium dihydrogen phosphate, pH 7.4, 0.05% Tween® 20
Qualiplate™ Kit for LibertyLink® PAT/*pat*	96-well plate precoated with anti-PAT antibody, PAT antibody/enzyme conjugate, substrate solution

PAT Extraction

PBST buffer was added to samples at a ratio of 3 ml of buffer to 15 mg of sample. The samples were mixed, homogenized in an Omni-Prep Multi-Sample Homogenizer, placed on wet ice to incubate for at least an hour, and centrifuged at 2°C to 8°C to form a pellet. The supernatants were removed and either analyzed the same day or stored at -20°C ± 5°C prior to analysis.

PAT Quantification

The appropriate number of 96-well plates pre-coated with the capture antibody, the appropriate amounts of PAT antibody/enzyme conjugate, and substrate solution were removed from storage at 2°C to 8°C and allowed to equilibrate to room temperature (all aforementioned items are provided in the Qualiplate™ ELISA Kit for LibertyLink® PAT/*pat*). The tube containing the substrate solution was covered to prevent exposure to light. The PAT enzyme conjugate solution was applied to each well at a volume of 50 µl/well. Immediately following the addition of the PAT antibody/enzyme conjugate solution, dilutions of each sample extract and the ELISA standard (prepared using protein reference substance, PAT-0109 [Seastrum 2009]), prepared in PBST buffer, were added to the pre-coated plates (50 µl/well). The plates were mixed in a rapid circular motion on the benchtop for 10 seconds and incubated at room temperature for at least one hour. The plates were washed five times with PBST buffer and the substrate solution was applied (100 µl/well). The plates were incubated at room temperature in the dark for 15 minutes. The colormetric reaction was stopped by the addition of 1N hydrochloric acid (100 µl/well) and absorbance was measured using a spectrophotometer at 450 nm and 650 nm. The results were analyzed with Molecular Devices SoftMax Pro® GxP Microplate Data Compliance Software, v. 5.4.1. The 650-nm reference measurement was subtracted from the 450-nm measurement prior to further analysis. Concentrations were interpolated from a standard curve generated using a quadratic curve fitting algorithm.

Validation of PAT ELISA Extraction Efficiency and Sensitivity

Method sensitivity (minimum dilution factor, limit of detection [LOD], and limit of quantitation [LOQ]) for soybean meal was determined and is summarized below. Extraction efficiency for soybean meal was not determined as PAT was not detected.

Minimum dilution factor. The minimum dilution factor for each sample type was determined by analyzing a dilution series of nontransgenic extracts spiked with a known quantity of PAT reference protein. The most concentrated dilution of spiked sample extract that yielded a percent recovery between 70% and 120%, and was followed by two subsequent dilutions with recoveries in the same range was selected as the minimum acceptable dilution factor.

Limit of detection. The LOD for each sample type was evaluated by comparison of the mean optical density (OD) plus three standard deviations of the unspiked nontransgenic sample extract with the mean OD of the nontransgenic sample extract spiked with PAT reference protein. The measured LOD is the lowest spike concentration with an OD greater than the mean OD plus three standard deviations of the unspiked nontransgenic sample extract.

The LOD (micrograms per gram of sample) was calculated by the following formula:

$$\left(\frac{\text{LOD (ng/ml)} \times \text{dilution factor} \times \text{volume of extraction buffer (ml)}}{\text{amount of tissue extracted (g)}}\right) \div 1000$$

Limit of quantitation. The LOQ for each sample type was evaluated by spiking nontransgenic sample extracts with known concentrations of PAT reference protein, and measuring the percent recovery of PAT protein. The LOQ was the lowest spike concentration of PAT that recovered between 70% and 120% of nominal value and was greater than or equal to the LOD.

The percent recovery for each spiked sample was calculated by the following formula:

$$\left(\frac{\text{mean protein concentration of spiked extract (ng/ml)}}{\text{spiked protein concentration (ng/ml)}}\right) \times 100$$

The LOQ (micrograms per gram of sample) was calculated by the following formula:

$$\left(\frac{\text{LOQ (ng/ml)} \times \text{dilution factor} \times \text{volume of extraction buffer (ml)}}{\text{amount of tissue extracted (g)}}\right) \div 1000$$

Extraction efficiency. Extraction efficiency from soybean meal was not determined as PAT was not detected.

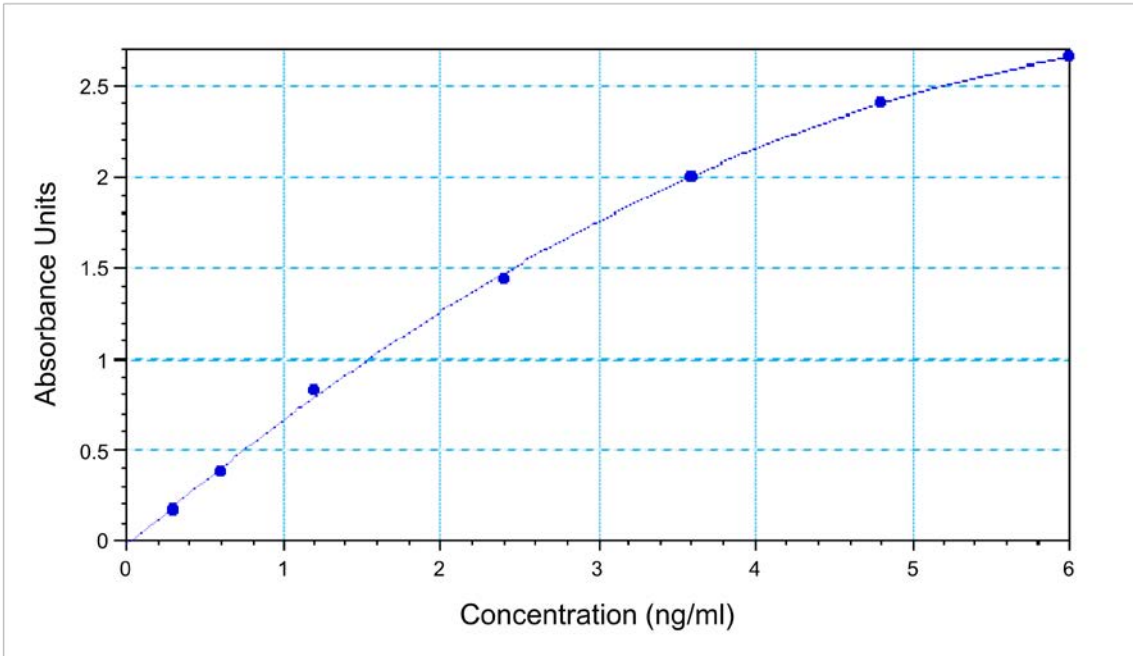
Method sensitivity data are summarized in the following table.

Minimum dilution factor, LOD, LOQ and extraction efficiency for each matrix

Sample Type	Minimum Dilution Factor	LOD (µg/g) FW	LOQ (µg/g) FW	Extraction Efficiency (%)
Soybean meal	1	0.05	0.05	–[a]

[a] Not determined because amount measured was less than LOD

Representative Standard Curve. Concentrations used to produce the ELISA standard curve were 6, 4.8, 3.6, 2.4, 1.2, 0.6, 0.250, and 0 ng/ml. A representative standard curve for the PAT ELISA is shown below.



References

Seastrum L. 2009. *Characterization of Microbially Produced Test Substance Containing Phosphinothricin Acetyltransferase (PAT) and Certificate of Analysis.* Report No. SSB-042-09 (unpublished). Research Triangle Park, NC: Syngenta Biotechnology.

APPENDIX C Event-Specific Polymerase Chain Reaction (PCR) Analysis

Genomic Deoxyribonucleic Acid (DNA) Extraction

Genomic DNA for real-time PCR analyses was isolated from soybean meal and broiler chicken diets by an extraction method adapted from the Wizard® Magnetic 96 DNA Plant System (Promega Corp., Madison, Wisconsin, USA). The supernatants were analyzed the same day.

Real-Time PCR

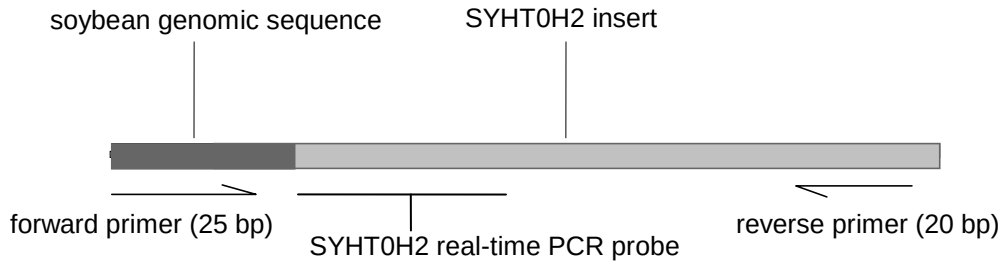
Genomic DNA extracted from SYHT0H2 soybean meal and broiler diets containing SYHT0H2 soybean meal was analyzed in duplicate for the presence of the SYHT0H2 insert by real-time PCR (Ingham *et al*. 2001). A control assay targeting a soybean native alcohol dehydrogenase 1 (*adh1*) gene, referred to as *GmADH* in this study, was included to monitor soybean DNA quality and performance of PCR components (*i.e.,* buffers, reagents, equipment, *etc.*). The sequences of the primers and probes are shown in the table below. The forward primer binding site is located in the soybean genomic sequence, the reverse primer binding site is located in the SYHT0H2 insert, and the probe binding site is located in the SYHT0H2 insert as shown in the following figure.

The following cycling parameters were used for this reaction: 95°C for five minutes, followed by 40 cycles of 95°C for five seconds and 60°C for 30 seconds.

Primers and probes used for detection of the SYHT0H2 insert and *GmADH1*

Amplicon of interest	Forward primer 5` to 3`	Reverse primer 5` to 3`	Probe 5` to 3`
SYHT0H2 insert	GGGAATTGGGTACCATGCC	TGTGTGCCATTGGTTTAGGGT	CCAGCATGGCCGTATCCGCAA
GmADH1	AGGTGTGGATCGGGCTGTT	CATCGTGGACGCATTCGA	ACTGGCAGCATCCAAGCCATGGTCT

Location of the Event SYHT0H2 real-time, event-specific PCR primer and probe binding sites



bp = base pair

References

Ingham DJ, Beer S, Money S, Hansen G. 2001. Quantitative real-time PCR assay for determining transgene copy number in transformed plants. *BioTechniques* 31:132–140.