7.5 Statistical Methods

No statistical analysis was performed.

8.0 Control of Bias

Measures taken to control bias in this study were the inclusion of both stability and test system experimental controls to account for any effects due to the model in the absence of the pancreatin enzyme and the absence of the test substance. Digestion specimens and lower limit of detection samples were analyzed concurrently to eliminate run-to-run variation.

9.0 Rejected Data

One set of data, which included determination of pepsin activity, *in vitro* digestibility of Cry2Ab2 in SGF, SDS-PAGE and western blot analysis of Cry2Ab2 digestions, was rejected because it was generated before the protocol was amended to include vortexing of the digestion tube throughout the duration of the digestion to improve exposure of the test protein to the test system.

10.0 Protocol Amendment

Section 6.1, describing digestibility of the test substance in SGF, was amended to address the possibility of test substance precipitation and/or aggregation at low pH. There was no negative impact on the study as a result of these changes.

11.0 Results and Discussion

11.1 Pepsin Activity in SGF

To assess the suitability of the test system used in this study, the pepsin activity in SGF was evaluated before conducting the digestion trials. Acceptable activity was defined as a pepsin activity per mg of solid equal to the activity of pepsin per mg of solid as determined by the manufacturer (±1000 units). The experimentally observed activity was 2429 units per mg pepsin powder, which was within the acceptable interval of pepsin activity (2280 to 4280) units per mg pepsin powder. Therefore, the test system was shown to be suitable for use in this study.

11.2 Assessment of Digestibility by Colloidal Brilliant Blue G Gel Staining of SDS-PAGE

The digestibility of the Cry2Ab2 protein was evaluated by visual analysis of colloidal Brilliant Blue G stained polyacrylamide gels (Figure 1). The SDS-PAGE for the digestibility assessment (Figure 1A) was run concurrently with a

separate SDS-PAGE to determine the LOD of Cry2Ab2 protein (Figure 1B). The limit of detection of full-length (~65 kDa) Cry2Ab2 was visually estimated to be 0.005 µg or approximately 0.6% of the total protein loaded:

$$\frac{0.005\,\mu g \times 100\%}{0.8\,\mu g} \cong 0.6\%$$

The gel used to assess the stability of the Cry2Ab2 protein to pepsin (Figure 1A) was loaded with ~0.8 µg total protein (based on pre-digestion concentrations) for each of the digestion time points. Visual examination of the stained gel showed that the full-length Cry2Ab2 protein was digested within 30 seconds of digestion in SGF (Figure 1A, lane 5). Therefore, at least 99.4% (100% - 0.6% = 99.4%) of the full-length Cry2Ab2 protein was digested within 30 seconds of incubation in SGF based on the Colloidal Brilliant Blue G Gel stained SDS-PAGE analysis. A faint band with molecular weight of ~5 kDa was observed at the 30-second digestion time point. No Cry2Ab2 bands were visible at the 2-minute digestion time point (Figure 1A, lane 6).

No change in the full-length Cry2Ab2 protein band intensity was observed in the absence of pepsin in the experimental controls P0 and P7 (Figure 1A, lanes 3 and 12). This indicates that digestion of the Cry2Ab2 protein was due to the proteolytic activity of pepsin present in SGF and not due to the instability of the test substance at pH ~1.3 and ~37°C.

The experimental controls evaluating the stability of the pepsin in the test system (SGF) lacking the test substance demonstrated that pepsin was observed as the stained protein band at ~38 kDa throughout the experimental phase (Figure 1A, lanes 2 and 13). The amount of pepsin slightly decreased between 30 and 60 min of the digestion, most probably due to enzyme auto-digestion.

11.3 Assessment of Digestibility by Western Blot Analysis

The digestibility of the Cry2Ab2 protein was also evaluated using western blotting (Figure 2). The western blot used to assess the stability of the Cry2Ab2 protein to pepsin digestion (Figure 2A) was run concurrently with a western blot to determine the LOD of Cry2Ab2 protein (Figure 2B). The LOD of full-length Cry2Ab2 was visually estimated to be 0.2 ng or 1% of the total protein loaded:

$$\frac{0.2\,ng \times 100\%}{20\,ng} = 1\%$$

The gel to assess the Cry2Ab2 protein *in vitro* digestibility by western blot was loaded with 20 ng total protein of the test substance (based on pre-digestion

concentrations) for each of the digestion time points. Western blot analysis demonstrated that the Cry2Ab2 protein was digested below the LOD within 30 seconds of incubation in SGF (Figure 2A, lane 5). Based on the western blot LOD for the Cry2Ab2 protein in SGF and the observation that no full-length protein or immunoreactive proteolytic bands were observed on the western blot at the 30-second digestion time point, it was concluded that at least 99% $(100\% - 1\% = 99\%)$ of the Cry2Ab2 protein was digested within 30 seconds.

No change in the full-length Cry2Ab2 protein band intensity was observed in the absence of pepsin in the experimental controls P0 and P7 (Figure 2A, lanes 2 and 12). This indicates that the test substance was stable in the test system without pepsin at pH ~1.3 and ~37 °C over the course of the experiment.

No immunoreactive bands were observed in specimens N0 and N7 that represent test system experimental controls (Figure 2A, lanes 3 and 13). This indicates that non-specific interactions between the test system components and the antibodies were not observed under these experimental conditions.

12.0 Conclusions

The results of this study demonstrated that the full-length Cry2Ab2 protein was rapidly digested after incubation in SGF. At least 99.4% of the full-length Cry2Ab2 protein was digested within 30 seconds when analyzed using Colloidal Brilliant Blue G stained polyacrylamide gels. At least 99% of the Cry2Ab2 protein was digested in SGF within 30 seconds when analyzed using western blot analysis. A faint proteolytic band of ~5 kDa was observed only at the 30-second digestion time point when analyzed using a stained polyacrylamide gel. No stable proteolytic bands were observed at any time points by western blot analysis.

13.0 References

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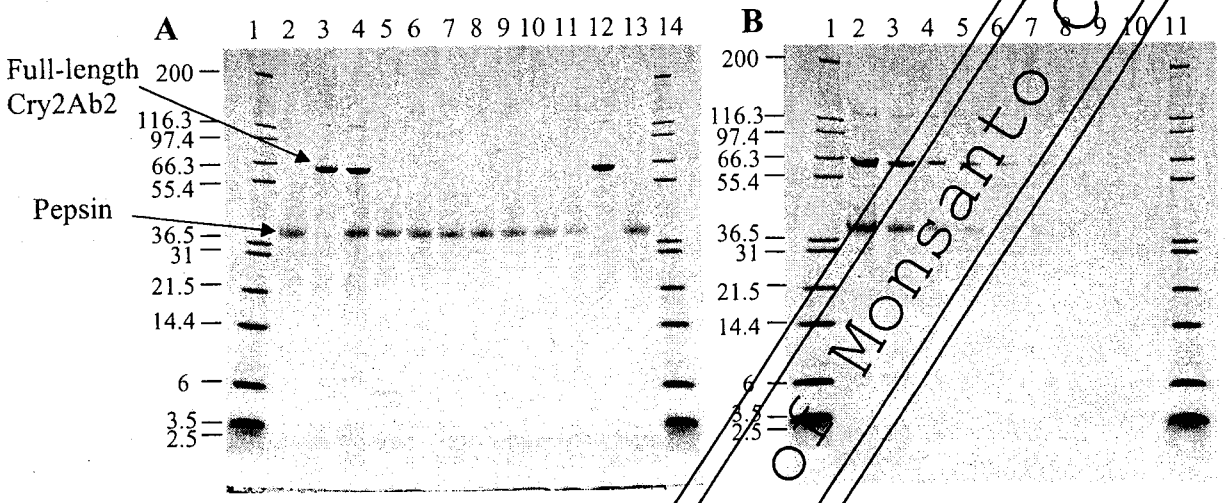
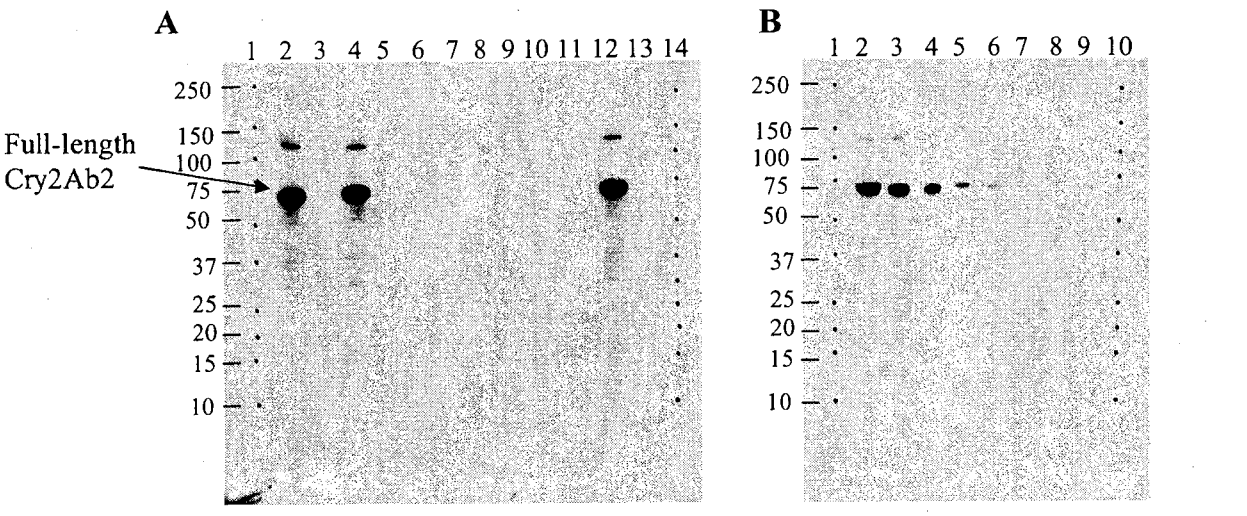


Lane	Sample	Incubation Time (min)
1	Molecular weight marker	n/a
2	N0, SGF only	0
3	P0, protein only	0
4	T0, protein+SGF	0
5	T1, protein+SGF	0.5
6	T2, protein+SGF	2
7	T3, protein+SGF	5
8	T4, protein+SGF	10
9	T5, protein+SGF	20
10	T6, protein+SGF	30
11	T7, protein+SGF	60
12	P7, protein only	60
13	N7, SGF only	60
14	Molecular weight marker	n/a

Lane	Sample	Amount (ng)
1	Molecular weight marker	n/a
2	T0, protein+SGF	1
3	T0, protein+SGF	0.5
4	T0, protein+SGF	0.2
5	T0, protein+SGF	0.1
6	T0, protein+SGF	0.05
7	T0, protein+SGF	0.02
8	T0, protein+SGF	0.01
9	T0, protein+SGF	0.005
10	T0, protein+SGF	0.0025
11	Molecular weight marker	n/a

Figure 1. Colloidal Brilliant Blue G stained SDS-Polyacrylamide Gels
Panel **A** corresponds to Cry2Ab2 protein digestion in SGF. Based on the pre-digestion protein concentration, ~0.8 µg (total protein) was loaded in lanes containing Cry2Ab2 protein. The incubation times are indicated. Panel **B** corresponds to the limit of detection of Cry2Ab2 protein. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in each gel. In both gels, Cry2Ab2 protein migrated to approximately 65 kDa (indicated by the arrow on the left). Blank lanes were cropped and lanes renumbered.



Lane	Sample	Incubation Time (min)
1	Molecular weight marker	n/a
2	P0, protein only	0
3	N0, SGF only	0
4	T0, protein+SGF	0
5	T1, protein+SGF	0.5
6	T2, protein+SGF	2
7	T3, protein+SGF	5
8	T4, protein+SGF	10
9	T5, protein+SGF	20
10	T6, protein+SGF	30
11	T7, protein+SGF	60
12	P7, protein only	60
13	N7, SGF only	60
14	Molecular weight marker	n/a

Lane	Sample	Amount (ng)
1	Molecular weight marker	n/a
2	T0, protein+SGF	10
3	T0, protein+SGF	5
4	T0, protein+SGF	2
5	T0, protein+SGF	1
6	T0, protein+SGF	0.5
7	T0, protein+SGF	0.2
8	T0, protein+SGF	0.1
9	T0, protein+SGF	0.05
10	Molecular weight marker	n/a

Figure 2. Western Blot Analysis
Panel **A** corresponds to Cry2Ab2 protein digestion in SGF. Based on the pre-digestion protein concentration, 20 ng (total protein) was loaded in lanes containing Cry2Ab2 protein. The incubation times are indicated. Panel **B** corresponds to the limit of detection of the Cry2Ab2 protein. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in each gel. In both gels, Cry2Ab2 migrated to approximately 65 kDa. A 10 min exposure is shown. Blank lanes were cropped and lanes renumbered.

Appendix. **List of Applicable SOPs**

BR-ME-0388-02	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
BR-ME-0392-01	Western Blot Analysis (Immunoblotting)
BR-ME-0460-02	Assay for Pepsin Activity in Simulated Gastric Fluid
BR-ME-0527-01	Brilliant Blue G-Colloidal Staining of Polyacrylamide Gels
BR-ME-0924-01	Electrotransfer of Proteins to Membranes
BR-ME-0973-01	Drying of Polyacrylamide Mini Gels Using Invitrogen Gel Drying System (Adaptation of Invitrogen Gel Drying Procedure)
BR-EQ-0599-02	Bio-Rad GS-710 and GS-800 Densitometers
BR-EQ-0857-01	Beckman Coulter DU-650 Spectrophotometer

Title

Assessment of the *in vitro* Digestibility of the Cry2Ab2 Protein in Simulated Intestinal Fluid

Author

Jamie J. Thorp
Michael E. Goley

Study Completed On

February 28, 2006

Performing Laboratory

Monsanto Company
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Laboratory Project ID

MSL-19938

Study 05-01-62-05

The text below applies only to use of the data by the United States Environmental Protection Agency (US EPA) in connection with the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

Statement of No Data Confidentiality Claim

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(l)(A), (B), or (C).

We submit this material to the United States Environmental Protection Agency specifically under the requirements set forth in FIFRA as amended, and consent to the use and disclosure of this material by EPA strictly in accordance with FIFRA. By submitting this material to EPA in accordance with the method and format requirements contained in PR Notice 86-5, we reserve and do not waive any rights involving this material that are or can be claimed by the company notwithstanding this submission to EPA.

Company: Monsanto Company

Company Agent: _____

Title: _____

Signature: _____ Date: _____

Statement of Compliance

This study meets the US EPA Good Laboratory Practice standards requirements as specified in 40 CFR Part 160.

Submitter: _____ Date: _____

Sponsor Representative: Scott Huber Date: 2/27/06

Study Director: Jami Shi Date: 2/28/06

Quality Assurance Statement

Study Title: Assessment of the *in vitro* Digestibility of the Cry2Ab2 Protein in Simulated Intestinal Fluid

Study Number: 05-01-62-05

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 of the study.

Following is a list of reviews conducted by the Monsanto Regulatory Quality Assurance Unit on the study reported herein.

Dates of Inspection / Audit	Phase	Date Reported To:	
		Study Director	Management
8/30/2005	Digestive Fate Inspection	9/1/2005	9/1/2005
9/1/2005	Western Blot Inspection	9/1/2005	9/1/2005
12/05/2005	Raw Data Audit	12/19/2005	12/19/2005
12/05/2005	Draft Report Review	12/19/2005	12/19/2005

Joan M. Reyda-Heath

Feb. 27, 2006
Date

Quality Assurance Unit
Monsanto Regulatory, Monsanto Company

Study Information

Study Number:	05-01-62-05
Title:	Assessment of the *in vitro* Digestibility of the Cry2Ab2 Protein in Simulated Intestinal Fluid
Primary Testing Facility:	Monsanto Company Product Characterization Center 800 North Lindbergh Boulevard St. Louis, MO 63167
Study Director:	Jamie J. Thorp Monsanto Company - U4A Product Characterization Center 800 North Lindbergh Boulevard St. Louis, Missouri 63167 Phone: (314) 694-5896
Principal Investigator:	Michael E. Goley Monsanto Company - U4A Product Characterization Center 800 North Lindbergh Boulevard St. Louis, Missouri 63167 Phone: (314) 694-6006
Record Retention:	All study specific raw data, protocol, final report, and facility records will be retained at Monsanto, St. Louis.
Specimens Retention:	Specimens will be retained at Monsanto, St. Louis, as specified in section 5.2.
Study Initiation Date:	August 15, 2005
Study Completion Date:	February 28, 2006

Study Certification

The results reported in this final report accurately reflect the data generated under study number 05-01-62-05.

Approved By:

Jamie J. Thorp
Study Director
Monsanto Company
Product Characterization Center

2/28/06
Date

Gary A. Bannon, Ph.D.
Protein Sciences Team Lead
Monsanto Company
Product Characterization Center

Feb 27, 2006
Date

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Appendix

Abbreviations and Definitions[1]

APS	Analytical Protein Standard
CAPS	3-(Cyclohexylamino)-1-propanesulfonic acid
CEW	Corn earworm
COA	Certificate of Analysis
DTT	DL-Dithiothreitol
EC_{50}	Effective protein concentration to inhibit the growth of the target insect by 50%
ECL	Enhanced chemiluminescence
E. coli	*Escherichia coli*
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
LB	Loading Buffer
LOD	Limit of detection
MALDI-TOF MS	Matrix assisted laser desorption and ionization - time of flight mass spectrometry
NFDM	Non-fat dry milk
PBST	Phosphate buffered saline containing Tween-20
PVDF	Polyvinylidene difluoride
SIF	Simulated intestinal fluid containing pancreatin
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
SOP	Standard operating procedure
TCA	Trichloroacetic acid
U	Unit (of enzyme activity)

[1] Standard abbreviations, e.g., units of measure, concentration, mass, time etc., are used without definition according to the format described in "Instructions to Authors" in The Journal of Biological Chemistry.

1.0 Summary

Monsanto has developed plants that produce the Cry2Ab2 insecticidal protein and are protected from feeding damage caused by European corn borer (*Ostrinia nubilialis*) and other lepidopteran insect pests. Cry2Ab2 is a *Bacillus thuringiensis* (subsp. *kurstaki)* protein.

The purpose of this study was to assess the *in vitro* digestibility of the Cry2Ab2 protein in simulated intestinal fluid (SIF), which contains a mixture of proteolytic enzymes known as pancreatin. The Cry2Ab2 protein used in this study was produced in and purified from *E. coli.* Digestibility in SIF was assessed using western blot analysis.

The results of this study demonstrated that at least 97.5% of the full-length Cry2Ab2 protein was digested within 15 min of incubation in SIF, yielding bands with molecular weights of approximately 60, 55, 50, 40, 12, and 10 kDa. The ~60 kDa band was undetectable at the 1-hour incubation time point. The ~55 kDa band was undetectable at the 24-hour incubation time point. The ~50 kDa band decreased over the time course but was still present at the 24-hour incubation time point. The ~ 40 kDa band was undetectable at the 12-hour incubation time point. The ~12 kDa bands, which migrated as a triplet, were detectable at the 5-minute incubation time point but were undetectable at the 1-hour incubation time point. Small bands that migrated as a doublet at ~10 kDa, which had a very weak immunoreactive signal, were detectable at various incubation time points. Several new bands with molecular weights smaller than 50 kDa were detectable beginning at the 4-hour incubation time point. These bands, which were transient in nature and displayed a weak immunoreactive signal, were present at the 24-hour incubation time point

2.0 Introduction

Monsanto has developed plants that produce the Cry2Ab2 insecticidal protein and are protected from feeding damage caused by European corn borer (*Ostrinia nubilalis*) and other lepidopteran insect pests. Cry2Ab2 is a *Bacillus thuringiensis* (subsp. *kurstaki*) protein.

Potential allergenicity and toxicity of the introduced protein can be evaluated using various methods, including digestibility of the protein by various proteases in simulated gastric and intestinal fluid assays (Astwood et al., 1996). SIF is an *in vitro* digestion model where proteins undergo digestion at neutral pH by a mixture of proteases known as pancreatin. The relationship between protein allergenicity and protein stability in the *in vitro* SIF study is limited, because the protein has not been first exposed to the acidic denaturing conditions of the stomach, as would be the case *in vivo* (FAO/WHO, 2001).

3.0 Purpose

The purpose of this study was to assess the stability of the Cry2Ab2 protein in simulated intestinal fluid containing a mixture of proteolytic enzymes called pancreatin.

4.0 Materials

4.1 Test Substance

The test substance is the Cry2Ab2 protein. The Cry2Ab2 protein (Analytical Protein Standard lot 20-100071) was isolated from a fermentation batch of *E. coli* containing the pMON70520 expression plasmid and is referred to as Cry2Ab2.820 on the Certificate of Anlaysis (COA). This protein has been characterized and has a total protein concentration of 0.5 mg/ml and a purity of 87 %. The activity was confirmed using an insect bioassay with the larvae of a sensitive pest, corn earworm (CEW), *Helicoverpa zea*. The EC_{50} value was 0.25 µg/ml of diet. Prior to its application to the test system, the test substance was stored in a -80 °C freezer in a storage buffer containing 50 mM CAPS, pH 11, and 2 mM DTT.

4.2 Control Substance

There was no control substance in this study.

4.3 Reference Substance

Analytical reference standards (e.g., molecular weight markers for SDS-PAGE) used in this study were documented in the data and are described in this report.

4.4 Characterization of Test Substance

The characterization of the physicochemical and functional properties of the test substance, Cry2Ab2 protein, was performed under characterization plan 20-100071 and is summarized on the Certificate of Analysis. Under this plan, the following properties were established for the Cry2Ab2 protein: identity (N-terminal sequencing, MALDI-TOF mass spectrometry (MS), immunoblot), concentration (amino acid analysis), purity (SDS-PAGE/densitometry), molecular weight (SDS-PAGE/densitometry, MALDI-TOF MS), short-term stability (SDS-PAGE/densitometry), and activity (CEW bioassay).

5.0 Test System

The test system for this study was SIF, which contains a mixture of proteolytic enzymes known as pancreatin. Normal proteolytic digestion of consumed food proteins starts with pepsin-mediated hydrolysis in the acidic environment of the stomach, and continues with neutral pH enzymatic digestion in the small intestine. The denaturing environment of the stomach (low pH) alters protein characteristics and facilitates proteolytic digestion in the small intestine by pancreatic enzymes. The physiological relevance of protein digestion in this *in vitro* SIF study is limited because these experimental conditions do not first

expose the protein to the acidic denaturing conditions of the stomach, as would be the case *in vivo*.

SIF was prepared according to the method described in The United States Pharmacopoeia (1995). The pancreatin enzyme used for the preparation of SIF was obtained from Sigma Company (Catalog number P1500, St. Louis, MO). The SIF was formulated so that 55.3 µg of pancreatin powder per 1 µg of test substance (total protein) would be present in the digestion reactions. The SIF was prepared by adding pancreatin powder (1.00 g) to 100 ml (final volume) of 50 mM monobasic potassium phosphate solution adjusted to pH 7.5 with sodium hydroxide. Activity of the SIF was assessed using an SIF activity assay. The assay was used to confirm activity before initiation of the digestion of the test substance.

5.1 Justification for Selection of the Test System

In vitro digestion models are widely used to assess the digestibility of ingested substances and SIF has been used for *in vitro* studies to assess the digestion of food components (Yagami et al., 2000; Okunuki et al., 2002).

5.2 Specimens

Specimens were generated by incubating the test substance with the test system for the times specified in section 5.3. See sections 6.0 and 7.2 for details on the preparation and analysis of specimens. Specimens will be retained in a –80°C freezer for one year from the end of study after which they will no longer afford analytical evaluation and may be discarded.

5.3 Procedure for Identification of Specimens

Alphanumerical codes were used to distinguish incubation time points and specimens (where T = time, P = protein only, and N = no protein):

Targeted Incubation Time Point	Code(s)
0 min	T0, P0, N0
5 min	T1
15 min	T2
30 min	T3
1 h	T4
2 h	T5
4 h	T6
8 h	T7
12 h	T8
24 h	T9, P9, N9

6.0 Experimental Design

6.1 Digestibility of the Test Substance in SIF

Digestion of the test substance in SIF was evaluated over time by analyzing specimens from all incubation time points. The target digestion temperature was 37 ± 2°C.

The digestion was prepared by adding 400 µl of the test substance to a tube containing 1.11 ml of SIF. The tube contents were vortex mixed and immediately placed in a 37 ± 2°C water bath. Digestion specimens (100 µl) of the digestion mixture were removed at 5, 15, and 31 min (specimens T1, T2, and T3, respectively), and 1, 2, 4, 8, 12, and 24 hours (specimens T4 to T9, respectively). Each 100 µl sample was immediately quenched by adding 25 µl of 5× LB [5× LB, 312.5 mM Tris-HCl, 25% (v/v) 2-mercaptoethanol, 10% (w/v) sodium dodecyl sulfate, 0.025% (w/v) Bromophenol Blue, and 50% (v/v) glycerol, pH 6.8] and heating to 75-100°C for 5-10 min.

The zero incubation time point (T0) was prepared in a separate tube by first quenching 55.3 µl of SIF with 19 µl of 5× LB followed by heating to 75-100°C for 5-10 min prior to the addition of 20 µl of the test substance.

All specimens were frozen on dry ice and stored in a −80°C freezer until analysis.

6.2 Experimental Controls

Specimens of stability experimental controls were prepared to determine the inherent stability (i.e., not attributable to digestion) of the test substance. These controls consisted of the test substance in the test system (SIF) that lacks the pancreatin enzymes. Specimens from these controls were identified with the letter "P" and prepared in separate tubes. The zero incubation time point specimen (P0) was prepared by quenching test system buffer (55.3 µl) with 5× LB (19 µl), heating to 75-100° C for 5-10 minutes, and adding 20 µl of the test substance. The 24-hour incubation time point specimen (P9) was prepared by adding 20 µl of the test substance to the test system buffer (55.3 µl), placing in a 37 ± 2°C water bath for 24 hours, adding 19 µl of 5× LB, and heating at 75-100°C for 5-10 minutes.

Specimens of test system experimental controls were prepared to determine any non-specific interactions between the test system components and the antibodies during western blot analysis of the specimens. These controls consisted of the test system that did not contain the test substance. Specimens from these controls were identified with the letter "N" and prepared in separate tubes. The zero incubation time point specimen (N0) was prepared by quenching test system buffer (55.3 µl) with 5× LB (19 µl), heating to 75-100°C for 5-10 minutes, and adding 20 µl of the storage buffer. The 24-hour incubation time point specimen

(N9) was prepared by adding 20 µl storage buffer to 55.3 µl of the test system, heating to 37 ± 2°C for 24 hours, adding 19 µl of 5× LB, and heating to 75-100°C for 5-10 minutes.

All specimens from the controls were frozen on dry ice and stored in a –80°C freezer until analysis.

7.0 Analytical Methods

Prior to the use of the SIF, its activity was assessed using an SIF activity assay. The digestion of the Cry2Ab2 protein in SIF was assessed using western blot analysis. The LOD of the Cry2Ab2 protein was determined for the western blot.

7.1 SIF Activity Assays

The SIF activity assay was used to confirm the suitability of the test system before the test substance was applied. According to SOP BR-ME-0461-03, acceptable activity was defined as 11,000 ± 3,000 U/ml. One unit of pancreatin activity in this assay is defined as an increase in the absorbance at 574 nm of 0.001 per min at 37 ± 2°C.

This assay is based on the estimation of the amount of soluble peptide present in a trichloroacetic acid (TCA) solution after pancreatin digestion of resorufin-labeled casein (Roche Molecular Biochemicals, Manheim, Germany). Undigested resorufin-labeled casein is precipitated with TCA, and the amount of soluble peptide is estimated by measuring the absorbance at 574 nm. The amount of soluble peptide is directly proportional to the amount of proteolytic activity.

Solutions containing resorufin-labeled casein were incubated with 0.05× SIF for 15 min at 37.5 °C. Blank samples of deionized water in place of SIF were incubated. The reaction was quenched by addition of 5% (w/v) TCA to the SIF-containing samples and the blank samples. Soluble material recovered after centrifugation was neutralized by the addition of an assay buffer [500 mM Tris-HCl, pH 8.8], and the absorbance of the clarified SIF-containing samples and the blank samples (each in triplicate) was read at 574 nm using a Beckman DU-650 spectrophotometer. The activity of SIF was calculated using the following equation:

$$\frac{Mean\,Test_{A574\,nm} - Mean\,Blank_{A574\,nm}}{0.001 \times 15\,\mathrm{min} \times 0.1\,ml \times 0.05}$$

where 0.001 is the change in the absorbance at 574 nm per min at 37 ± 2°C produced by one unit of pancreatin activity, 15 min is the reaction time, 0.1 ml is the amount of 0.05× SIF added to the reaction, and 0.05 is a dilution factor.

7.2 Western Blot Analysis

Specimens from the Cry2Ab2 protein *in vitro* digestion in SIF were separated by SDS-PAGE using pre-cast 4-20% tris-glycine gradient mini-gels (Invitrogen, Carlsbad, CA) with tris-glycine SDS running buffer. The amount of protein loaded in each lane was based on pre-digestion concentrations of the Cry2Ab2 protein (total protein). The digestion specimens were diluted with 1× LB to a concentration of ~2.5 ng/µl and ~20 ng of total protein was loaded in each lane. The experimental controls specimens were loaded in the same volumes as the digestion specimens. All specimens were heated to 98°C for 5 min prior to loading on the gels. Electrophoresis using 4-20% tris-glycine gels was performed at 125 V for 90 min and 150 V for 15 min until the dye front reached the bottom of the gel. After electrophoresis, proteins were electrotransferred to a polyvinylidene difluoride (PVDF) membrane (Invitrogen) for 60 min at a constant voltage of 25 V. Pre-stained molecular weight markers (Precision Plus Dual Color, Bio-Rad, Hercules, CA) were used to verify electrotransfer of proteins to the membrane.

Proteins transferred to a PVDF membrane were analyzed by western blot. The membrane was blocked overnight at ~4 °C with 5% (w/v) non-fat dry milk (NFDM) in PBST buffer. All subsequent incubations (described below) were performed at room temperature. Goat anti-Cry2Ab2 antibody (lot 7227632) was incubated with the membrane for 60 min at a dilution of 1:3,000 in 1% (w/v) NFDM in PBST. Excess serum was removed by three 10 min washes with PBST. The membrane was incubated with HRP–conjugated rabbit anti-goat IgG (Sigma, St. Louis, MO) at a dilution of 1:10,000 in 1% (w/v) NFDM in PBST for 60 min, and again washed (three 10-min washes) with PBST. Immunoreactive bands were visualized using the enhanced chemiluminescence (ECL) detection system (Amersham Bioscience, Piscataway, NJ) and exposed to Hyperfilm ECL high performance chemiluminescence film (Amersham Biosciences). Films were developed using a Konica SRX101A automated film processor (Tokyo, Japan). The films were scanned using a Bio-Rad GS-800 densitometer to produce electronic images to be used as figures for reporting purposes.

The approximate molecular weights of the full-size protein and proteolytic bands observed on the western blot were visually determined relative to the positions of the molecular weight markers.

The limit of detection (LOD) for the western blot analysis procedure was determined for the Cry2Ab2 protein by loading various dilutions of the zero incubation time point (T0) specimen onto a separate gel. This gel was run concurrently with the digestion western blot gel and subjected to the same western blot procedure as described above. The following approximate total protein loadings of the T0 specimens were used for the western blot LOD analysis: 0.5, 1, 2, 5, 10, and 20 ng.

7.3 Statistical Methods

No statistical analysis was performed.

8.0 Control of Bias

Measures taken to control bias in this study were the inclusion of both stability and test system experimental controls to account for any effects due to the model in the absence of the pancreatin enzyme and the absence of the test substance. Digestion specimens and LOD specimens were analyzed concurrently to eliminate run-to-run variation.

9.0 Results and Discussion

9.1 SIF Activity

In order to assess the suitability of the test system used in this study, the pancreatin activity in SIF was evaluated before the test system was applied. The experimentally observed activity was 10,760 U/ml and was within the acceptable interval of SIF activity (8,000 to 14,000 units per ml of SIF). Therefore, the test system was shown to be active and suitable for use in this study.

9.2 Assessment of Digestibility by Western Blot Analysis

The digestion of the Cry2Ab2 protein was evaluated by a western blot method, as described in Section 7.2 and shown in Figure 1 (5-minute exposure). A western blot to determine the LOD (Figure 1A) of the Cry2Ab2 protein was run concurrently with the western blot used to assess the Cry2Ab2 protein *in vitro* digestibility in SIF (Figure 1B). The LOD was determined by the lowest amount of Cry2Ab2 protein observed on the X-ray film under visual inspection. The LOD was visually estimated to be 0.5 ng, which represents 2.5% of the total protein loaded:

$$\frac{0.5\ ng \times 100\%}{20\ ng} = 2.5\%$$

The gel used to assess the Cry2Ab2 protein *in vitro* digestibility by western blot was loaded with 20 ng total protein of the test substance (based on pre-digestion concentrations) for each of the incubation time points. Western blot analysis demonstrated that a band corresponding to the full-length Cry2Ab2 protein was digested below the LOD within 15 minutes of incubation in SIF (Figure 1B, lane 6). Therefore, at least 97.5% (100% - 2.5% = 97.5%) of the full-length Cry2Ab2 protein was digested within 15 minutes. Proteolytic bands with approximate molecular weight of 60, 55, 50, 40, 12 and 10 kDa were observed at the 5-minute time point. The ~ 60 kDa band was undetectable at the 1-hour incubation time point. The ~ 55 kDa band decreased during the time course and was undetectable at the 24-hour time point. The ~ 50 kDa band decreased over the time course but still detectable at the 24-hour incubation time point. The ~ 40 kDa band was undetectable at the 12-hour incubation time point. The ~12 kDa bands, which migrated as a triplet, were detectable at the 5-minute incubation

time point but were undetectable at the 1-hour incubation time point. Small bands that migrated as a doublet at ~ 10 kDa, which had a weak immunoreactive signal, were detectable at various incubation time points. Several new bands with molecular weights smaller than 50 kDa were detectable beginning at the 4-hour digestion time point. These bands, which were transient in nature and displayed a weak immunoreactive signal, were detectable at the 24-hour incubation time point.

No change in the full-length Cry2Ab2 protein band intensity was observed in specimens P0 and P9 (Figure 1B, lanes 2 and 14) from the stability experimental controls. This indicates that the test substance was stable in the test system without pancreatin at ~37°C over the course of the incubation.

No immunoreactive bands were observed in specimens N0 and N9 (Figure 1B, lanes 3 and 15) from the test system experimental controls. This indicates that the test system components did not produce a non-specific interaction with the antibodies during the western blot analysis.

10.0 Conclusions

The results of this study demonstrated that at least 97.5% of the full-length Cry2Ab2 protein was digested within 15 minutes of incubation in SIF, yielding bands with molecular weights of approximately 60, 55, 50, 40, 12, and 10 kDa. These bands represent the digestion of Cry2Ab2 protein into smaller peptides during incubation in SIF. The bands with approximate molecular weights of 60, 55, 40, 12, and 10 kDa were undetectable at the 24-hour incubation time point. The ~50 kDa band decreased over the time course but was still present at the 24-hour incubation time point.

11.0 References

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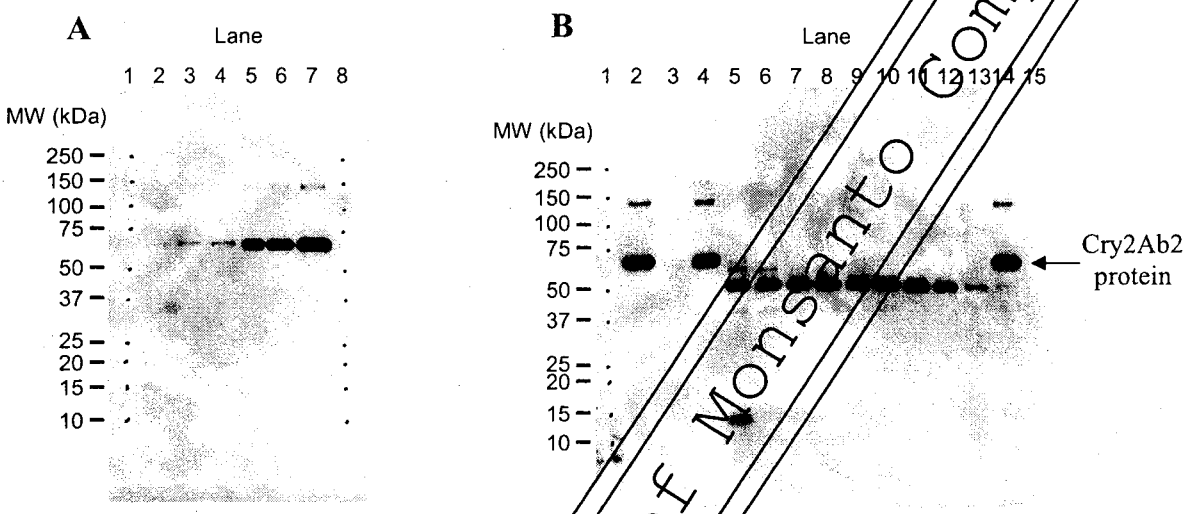

Lane	Sample	Amount (ng)
1	Molecular weight marker	—
2	T0, protein + SIF	0.5
3	T0, protein + SIF	1
4	T0, protein + SIF	2
5	T0, protein + SIF	5
6	T0, protein + SIF	10
7	T0, protein + SIF	20
8	Molecular weight marker	—

Lane	Sample	Incubation Time
1	Molecular weight marker	—
2	P0, protein only	0
3	N0, SIF only	0
4	T0, protein + SIF	0
5	T1, protein + SIF	5 min
6	T2, protein + SIF	15 min
7	T3, protein + SIF	30 min
8	T4, protein + SIF	1 h
9	T5, protein + SIF	2 h
10	T6, protein + SIF	4 h
11	T7, protein + SIF	8 h
12	T8, protein + SIF	12 h
13	T9, protein + SIF	24 h
14	P9, protein only	24 h
15	N9, SIF only	24 h

Figure 1. Western Blot Analysis

Figure 1A corresponds to the LOD of Cry2Ab2 protein (5-min exposure). Figure 1B corresponds to Cry2Ab2 protein digestion in SIF (5-min exposure). Based on the pre-digestion concentration, 20 ng (total protein) was loaded in lanes containing Cry2Ab2 protein. The incubation times are indicated. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded on each gel. In both gels, Cry2Ab2 protein migrated to ~ 65 kDa. Blank and empty lanes were cropped and lanes were renumbered in Figure 1A.

Appendix

List of Applicable SOPs

BR-ME-0388-02	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
BR-ME-0392-01	Western Blot Analysis (Immunoblotting)
BR-ME-0461-02	Assay for Proteolytic Activity in Simulated Intestinal Fluid
BR-EQ-0599-02	Bio-Rad GS-710 and GS-800 Densitometers
BR-EQ-0857-01	Beckman Coulter DU-650 Spectrophotometer
BR-ME-0924-01	Electrotransfer of Proteins to Membranes

Study Title

Amended Report for MSL-20097: Compositional Analyses of Corn Forage and Grain Collected from MON 89034 Grown in 2004 U.S. Field Trials

Authors

Tracey L. Reynolds, Ph.D.
Suzanne M. Drury
Margaret A. Nemeth, Ph.D.
***William A. Trujillo**
****Roy Sorbet**

Study Completed On

Amendment 1
September 15, 2006

Performing Laboratories

Monsanto Company
Product Safety Center
800 North Lindbergh Blvd.
St. Louis, MO 63167

***Covance Laboratories Inc.**
3301 Kinsman Blvd.
Madison, WI 53704

Statistical Analysis Facility

****Certus International, Inc.**
1422 Elbridge Payne Road, Suite 200
Chesterfield, MO 63017

Laboratory Project ID

MSL-20403

The text below applies only to use of the data by the United States Environmental Protection Agency (US EPA) in connection with the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

Statement of No Data Confidentiality Claim

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA10 (d), (1) (A), (B) or (C).

"We submit this material to the United States Environmental Protection Agency specifically under the requirements set forth in FIFRA as amended, and consent to the use and disclosure of this material by EPA strictly in accordance with FIFRA. By submitting this material to EPA in accordance with the method and format requirements contained in PR Notice 86-5, we reserve and do not waive any rights involving this material that are or can be claimed by the company notwithstanding this submission to EPA."

Monsanto Company

Company Agent

Title

Signature Date

Statement of Compliance

This study meets the requirements under GLP as specified in 40 CFR Part 160 (U.S. EPA) with the following exceptions:

- The reference standards used for compositional analysis were not characterized according to GLP standards and reserve samples from each batch of the reference standards were not retained. These exceptions had no effect on the integrity or quality of the study because the reference standards were accompanied by Certificates of Analysis.

- Stability of the compositional analytes in the test, control, and reference substances was not determined. This exception had no effect on the integrity or quality of the study because the samples were maintained at approximately –20°C throughout the duration of the study.

Although not specifically required for product characterization studies as defined by 160.135(b), this study fully complied with sections 160.35 (Quality Assurance), 160.120 (Protocol), and 160.185 (Reporting of Study Results). These elements were utilized to enhance the quality of this study.

Submitter	Date
	13-Sept-2006
Yong Gao, Ph.D. Sponsor Representative	Date
Tracey Reynolds	Sept 15/2006
Tracey L. Reynolds, Ph.D. Study Director	Date

Quality Assurance Statement

Study Title: Amended Report for MSL-20097: Compositional Analyses of Corn Forage and Grain Collected from MON 89034 Grown in 2004 U.S. Field Trials

Study Number: 05-01-50-09

Reviews conducted by the Quality Assurance Unit (QAU) confirm that the final report reflects the raw data for the portion of the study conducted by Monsanto Company, Biotechnology Regulatory Sciences.

Reviews which have been conducted by the Covance Laboratories Inc., are enclosed within the Covance sub-report and are specified on their individual QA Statement (see Appendix 2).

Following is a list of reviews conducted by the Monsanto Regulatory QAU on the study reported herein.

Dates of Inspection/Audit	Phase	Date Reported to Study Director	Date Reported to Management
02/01/2006	Raw Data and Draft Report Review	02/10/2006	02/10/2006
02/01/2006	Statistical Data and Draft Report Review	02/10/2006	02/10/2006
02/01/2006	Sub-Report Review	02/10/2006	02/10/2006
09/05/2006	Amended Report Audit	09/05/2006	09/05/2006

Kristin Berman — September 11, 2006

Quality Assurance Specialist
Monsanto Company

Date

Study Information

Study Number:	05-01-50-09
Study Title:	Amended Report for MSL-20097: Compositional Analyses of Corn Forage and Grain Collected from MON 89034 Grown in 2004 U.S. Field Trials
Primary Testing Facility:	Monsanto Company Product Safety Center 800 North Lindbergh Blvd. St. Louis, Missouri 63167
Performing Laboratories:	Covance Laboratories Inc. 3301 Kinsman Blvd. Madison, WI 53704 Certus International, Inc. 1422 Elbridge Payne Rd. Ste. 200 Chesterfield, MO 63017
Study Director:	Tracey L. Reynolds, Ph.D.
Sponsor Representative:	Yong Gao, Ph.D.
Principal Investigators:	William A. Trujillo (Covance Laboratories Inc) Roy D. Sorber (Certus International, Inc.)
Contributors:	Susan G. Riordan
Study Initiation Date:	May 18, 2005
Study Completion Dates: **Original Study** **Amended Study**	 January 5, 2006 September 15, 2006
Records Retention:	All study specific raw data, protocols, final reports and facility records will be retained at Monsanto, St. Louis except for analytical raw data and facility records maintained at Covance Laboratories Inc., Madison facility.
Sample Storage:	Any unused study samples will be stored at Covance Laboratories Inc. until their final disposition is directed by the Study Director at a future date.

Study Information (continued)

Amendment to Report MSL-20097

This amendment modifies the final report to reflect modifications to Tables 1 and 14, a discussion of results was added to address biological relevance of data, and both summary and conclusion sections were modified to reflect the results discussion. "Amendment 1" was added to the footer of each amended page and the new MSL # was added to the header of each page. These changes improve the accuracy of the report and had no impact on the study.

Item No.	MSL-20097 Original Report	MSL-20403 Amendment 1 Report	Amendment
1	Title Page	Title Page	Added "Amended Report for MSL-20097" to title, "Amendment 1" after "Study Completed on" and revised report completion date. MSL # changed to 20403
2	Page 2-4, 7	Page 2-4, 7	Added new signatures and dates
3	Page 3, 5	Page 3, 5	Sponsor Representative changes
4	Page 4	Page 4	Added "Amended Report for MSL-20097" to title; added "Amended Report Audit" and appropriate dates to list of phases
5	Page 5	Page 5	Added "Amended Report for MSL-20097" to title; added "Original Study" and "Amended Study" to study completion dates, and added amended study completion date
6	Page 6	Page 6	Added Amendment to Report MSL-20097, added list of changes, and added sponsor and study director with new signatures and appropriate dates
7	Page 7	Page 7	Sponsor Representative removed
8	Page 8-10	Page 8-10	Pagination changes and new title for Tables 1 and 14
9	Page 12-13	Page 12-13	Summary text modified per amended results discussion
10	Page 23-24	Page 23-25	Discussion of biological relevance of data added
11	Page 25	Page 25	Conclusion text modified per amended results discussion
12	Page 32-35	Page 32-35	New title and format change made to Table 1
13	Page 84-86	Page 84-86	New title and format change made to Table 14. ILSI values replaced historical values

Sponsor — Date: 13-Sept-2006

Study Director: Tracey Reynolds — Date: Sept 15/2006

Study Certification

This report is a true and accurate reflection of the work conducted in this study.

Signature of Final Report Approval:

Tracey Reynolds

Tracey L. Reynolds, Ph.D., Study Director

Sept 15/2006
Date

Table of Contents

Tables

Abbreviations

AA	Amino acid
AACC	American Association of Cereal Chemists
ADF	Acid detergent fiber
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists Society
B.t.	*Bacillus thuringiensis*
CI	Confidence interval
CRW	Corn rootworm
DW or dw	Dry weight
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase enzyme
FA	Fatty acid
FW or fw	Fresh weight
LOQ	Limit of quantitation
NDF	Neutral detergent fiber
OECD	Organization for Economic Co-operation and Development
PCR	Polymerase chain reaction
ppm	Parts per million
SE	Standard error
SOP	Standard operating procedure
T/C/R	Test/Control/Reference
TDF	Total dietary fiber

1.0 Summary

Monsanto Company has developed corn, MON 89034, that produce the Cry1A.105 and Cry2Ab2 insecticidal proteins and are protected from feeding damage caused by European corn borer (*Ostrinia nubilalis*) and other lepidopteran insect pests. Cry1A.105 is a modified *Bacillus thuringiensis* (*B.t.*) Cry1A protein. Cry2Ab2 is a *B.t.* (subsp. *kurstaki*) protein. The combination of the Cry2Ab2 and Cry1A.105 insecticidal proteins in a single plant provides better insect control and offers an additional insect-resistance management tool.

The purpose of this study was to evaluate the composition of MON 89034 compared to the conventional control corn. The test, control, and reference substances in this study were grown at five replicated field sites across the U.S. during the 2004 field season under Production Plan 04-01-50-02 (Leafgren, 2005). The conventional control substance used in this study, LH198 × LH172, has the genetic background representative of the test substances, MON 89034, but does not contain the Cry1A.105 and Cry2Ab2 proteins. Fifteen different conventional corn substances were included as references to provide data for the development of a 99% tolerance interval for each component analyzed.

Forage and grain samples were harvested from all plots and analyzed for compositional components. Analyzed components were selected based on recommendations specified by the OECD (OECD, 2002). Compositional analyses of the forage samples included proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals (calcium and phosphorus), and carbohydrates by calculation. Compositional analyses of the grain samples included proximates (protein, fat, ash, and moisture), ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), vitamins (B_1, B_2, B_6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-coumaric acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and carbohydrates by calculation. A total of 77 different analytical components (nine in forage and 68 in grain) were measured. Of these components, 16 had more than 50% of the observations below the assay LOQ and, as a result, were excluded from the statistical analysis. Therefore, 61 components were statistically assessed (nine in forage and 52 in grain). Statistical evaluation of the compositional data was conducted using a mixed model analysis of variance on six sets of data: analyses of data from each of the five replicated field trials plus data from a combination of all five field trials, referred to as the combined site in this report. Statistical evaluation of the composition data involved a comparison of the forage and grain from MON 89034 to a conventional control corn substance. Statistically significant differences were determined at the 5% level of significance ($p<0.05$). There were 366 statistical comparisons conducted between the test substance and the conventional control (61 comparisons in the combined site and 305 comparisons in the individual sites). Using the data for each component obtained from the

15 unique conventional substances, a 99% tolerance interval was calculated to contain, with 95% confidence, 99% of the values contained in the population of conventional corn substances. For those comparisons in which the test was significantly different ($p<0.05$) from the control, the test range was then compared to the 99% tolerance interval in order to determine if the test range was within the tolerance interval and, therefore, considered to be part of the population of the conventional corn.

Statistical analyses for MON 89034 from the combined site showed statistically significant differences for three analytes. For two of these analytes, there were also statistically significant differences in more than one of the individual sites. For the remaining one analyte, there was a statistical difference in only one of the individual sites. Statistical analyses for MON 89034 from the five individual sites showed that 11 analytes were observed to be statistically different from the control in more than one of the individual sites and 33 analytes were observed to be statistically different from the control in only one of the individual sites. All means and range of values from the test substance were within the range of values obtained from either the 99% tolerance interval, and/or the ILSI Crop Composition Database ranges, therefore these differences were not considered to be biologically relevant.

2.0 Introduction

Monsanto Company has developed corn, MON 89034, that produce the Cry1A.105 and Cry2Ab2 insecticidal proteins and are protected from feeding damage caused by European corn borer (*Ostrinia nubilalis*) and other lepidopteran insect pests. Cry1A.105 is a modified *Bacillus thuringiensis* (*B.t.*) Cry1A protein. Cry2Ab2 is a *B.t.* (subsp. *kurstaki*) protein. The combination of the Cry2Ab2 and Cry1A.105 insecticidal proteins in a single plant provides effective insect control and offers a valuable insect-resistance management tool.

3.0 Purpose

The purpose of this study was to evaluate the composition of the MON 89034 test substance. MON 89034 was compared to the conventional control substance and the conventional reference substances. The test, control, and reference corn substances in this study were grown at five replicated field sites across the U.S. during the 2004 field season under Production Plan 04-01-50-02 (Leafgren, 2005). The conventional control substance used in this study, LH198 × LH172, has the genetic background representative of the test substance, but does not contain the Cry1A.105 and Cry2Ab2 proteins. Fifteen different conventional corn substances were included as references to provide data for the development of a 99% tolerance interval for each component analyzed. Forage and grain samples were harvested from all plots and analyzed for compositional components. The

compositional and statistical results summarized in this report are based on the analytical and statistical data in the final report for MSL # 20402 (Reynolds et al., 2006).

4.0 Test, Control, and Reference (T/C/R) Substances

4.1 Test Substance

The test substance is described below. Forage and grain tissues of the test substance were evaluated in this study.

Description	Starting Seed Lot No.
MON 89034	GLP-0404-14916-S

4.2 Control Substance

The control substance is conventional corn with genetic background representative of the test substance and is described below. The forage and grain tissues of the control substance were evaluated in this study.

Description	Starting Seed Lot No.
LH198 × LH172	GLP-0404-14928-S

4.3 Reference Substances

The reference substances are conventional commercial corn samples and are described below. A single replicate of the forage and grain tissues from each reference substance was evaluated in this study.

Vendor/Hybrid	Starting Seed Lot No.	Field Site
Golden Harvest/ H8751	REF-0404-14931-S	IA
Golden Harvest/ H9231	REF-0404-14932-S	IA
Northrup King/ N60-N2	REF-0404-14933-S	IA
Burrus/ 590	REF-0404-14934-S	IL-1
Mycogen/ 2784	REF-0404-14935-S	IL-1
Dekalb/ DKC62-15	REF-0404-14936-S	IL-1
Pfister/ 2730	REF-0404-14937-S	IL-2
Mycogen/ 2E685	REF-0404-14938-S	IL-2

Dekalb/ DKC61-42	REF-0404-14939-S	IL-2
Dekalb/ DKC60-15	REF-0404-14940-S	NE
Mycogen/ 2P682	REF-0404-14941-S	NE
Mycogen/ 2A791	REF-0404-14942-S	NE
Seed Consultants / SC1124A	REF-0404-14943-S	OH
Crow's/ 4908	REF-0404-14944-S	OH
Asgrow/ RX708	REF-0404-14946-S	OH

4.4 T/C/R Substance Characterization

The identities of the test, control, and reference substances were verified by the Study Director prior to their use in the study by confirming the chain-of-custody documentation supplied with the samples collected from the field. The grain samples from the test, control, and reference substances were further characterized by an event-specific PCR analysis of DNA extracted from grain to confirm the presence or absence of each event. The presence or absence of MON 89034 in respective samples of the grain from the test and control substances were confirmed. All forage samples were characterized by the confirmation of chain-of-custody records. Characterization data were archived under Production Plan 04-01-50-02.

5.0 Field Trial Description

Forage and grain of the test, control, and reference substances were collected at five replicated field sites in the U.S. as detailed in Production Plan 04-01-50-02. Seed was planted in a randomized complete block design with three replicates per block of each test, control, and reference substance. All the samples at the field sites were grown under normal agronomic field conditions for their respective geographic regions. The five U.S. sites were: Site 1-Jefferson County, IA; Site 2-Jersey County, IL; Site 3-Warren County, IL; Site 4-York County, NE; and Site 5-Fayette County, OH. Forage and grain samples were harvested from all plots and shipped on dry ice (forage) or ambient temperature (grain) to Monsanto Company, St. Louis, MO, USA. A sub-sample for use in compositional analysis was obtained from each bulk forage and grain sample generated in the field. Each sub-sample was ground, stored in a -20°C freezer located at Monsanto Company (St. Louis, MO), and then shipped, overnight, on dry ice to Covance Laboratories, Inc. (Madison, WI) for analyses. The labels on the samples shipped to Covance Laboratories, Inc. listed the composition protocol number, a unique sample number, line/event number, tissue type, and storage conditions.

6.0 Analytical Methods

A total of 90 ground forage and grain samples were analyzed by Covance Laboratories Inc. Compositional analyses of the forage samples included proximates (protein, fat, ash, and moisture), ADF, NDF, minerals (calcium and phosphorus), and carbohydrates by calculation. Compositional analyses of the grain samples included proximates (protein, fat, ash, and moisture), ADF, NDF, TDF, amino acids, fatty acids, vitamins (B_1, B_2, B_6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-coumaric acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and carbohydrates by calculation. The methods used for compositional analyses are summarized below. The analytical data generated by Covance Laboratories, Inc., including a summary of the methods used, Covance SOP or method mnemonics, literature references, limits of quantitation, and the reference standards used, can be found in the final report of MSL # 20402 (Reynolds et al., 2006). The Study Director approved all methods utilized in this study.

6.1 Acid Detergent Fiber. The method used was based on an USDA Agriculture Handbook No. 379 (1970) method. The sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. An acetone wash was used to remove the fats and pigments. The lignocellulose fraction was collected on the frit and determined gravimetrically. The limit of quantitation of this method was 0.1% fw.

6.2 Amino Acid Composition. The method used was based on AOAC International (2000) method 982.30 that estimates the levels of 18 amino acids in the sample: alanine, arginine, aspartic acid (including asparagine), cystine (including cysteine), glutamic acid (including glutamine), glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis using sodium hydroxide. Sulfur-containing amino acids required an oxidation using performic acid prior to hydrolysis with hydrochloric acid. Analysis of the remaining amino acids was accomplished through direct hydrolysis with hydrochloric acid. The individual amino acids were quantitated using an automated amino acid analyzer. The limit of quantitation of this method was 0.1 mg/g fw. The reference standards were Beckman K18, 2.5 µmol/mL per constituent except cystine (1.25 µmol/mL), lot number S407158; Sigma L-Tryptophan, 100%, lot number 063K0382; Fluka L-Cysteic Acid Monohydrate, 100%, lot number 1157629; Sigma L-Methionine Sulfone, 100%, lot number 12H3349.

6.3 Ash. The method used was based on AOAC International (2000) method 923.03. The sample was placed in an electric furnace at 550 °C and ignited to drive off volatile organic compounds. The nonvolatile matter remaining was quantitated

gravimetrically and the percent ash was determined by calculation. The limit of quantitation of this method was 0.1% fw.

6.4 Carbohydrates. The method used was based on an USDA Agriculture Handbook No. 74 (1973) method. The limit of quantitation of this method was 0.1% fw. Carbohydrate values were calculated by difference using the fresh weight-derived data and the following equation:

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

6.5 Fat by Acid Hydrolysis. The method used was based on AOAC International (2000) methods 922.06 and 954.02. The forage sample was hydrolyzed with hydrochloric acid at an elevated temperature. The fat was extracted using diethyl ether followed by hexane. The extract was evaporated under nitrogen, re-dissolved in hexane and filtered through a sodium sulfate column. The hexane extract was then evaporated gagin under nitrogen, dried, and weighed. The limit of quantitation of this method was 0.1% fw.

6.6 Fat by Soxhlet Extraction. The method used was based on AOAC International (2000) method 960.39. The grain 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 evaporated, dried, and weighed. The limit of quantitation of this method was 0.1% fw.

6.7 Fatty Acids. The method used was based on AOCS (1997) method Ce 1-62 that estimates the levels of 22 fatty acids in the sample: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:0 palmitic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic, 18:3 gamma linolenic acid, 20:0 arachidic acid, 20:1 eicosenoic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, and 22:0 behenic acid. Lipid in grain samples was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated with 14% (weight/volume) boron trifluoride:methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The limit of quantitation of this method was 0.003% fw. The reference standards were Nu Chek Prep GLC reference standard Hazelton no. 1, used as 100%, lot number D13-0; Nu Chek Prep GLC reference standard Hazelton no. 2, used as 100%, lot number M13-0; Nu Chek Prep GLC reference standard Hazelton no. 3, used as 100%, lot number MA13-0; Nu Chek Prep GLC reference standard Hazelton no. 4, used as 100%, lot number D13-0; Nu Chek Prep methyl gamma linolenate, used as 100%, lot number U-63M-MA19-0; and Sigma methyl tridecanoate, used as 100%, lot number 035K1392.

6.8 Folic acid. The method used was based on AOAC International (2000) methods 960.46 and 992.05 and Methods of Analysis for Infant Formulas (1973), Section C-2. The grain sample was hydrolyzed in potassium phosphate buffer with the addition of ascorbic acid to protect the folic acid during autoclaving. Following hydrolysis, the sample was treated with a chicken-pancreas enzyme and incubated approximately 18 hours to liberate the bound folic acid. The amount of folic acid was turbidimetrically determined by comparing the growth response of the bacteria *Lactobacillus casei* in the sample versus the growth response in folic acid standard. The limit of quantitation of this method was 0.06 µg/g fw. The reference standard was USP folic acid 98%, lot number P.

6.9 Furaldehyde. The method used was based on a literature method (Albala-Hurtado et al., 1997). The ground grain sample was extracted with 4% trichloroacetic acid. The level of 2-furaldehyde (furfural) in the extract was determined by HPLC with UV quantitation. The reference standard was ACROS 2-furaldehyde, 99%, lot number A018806701. The quantitation limit of this method was calculated to be 0.5 ppm.

6.10 Minerals/ICP emission spectrometry. The method used was based on AOAC International (2000) methods 984.27 and 985.01 and a literature method (Dahlquist and Knoll, 1978). Samples were dried, precharred, and ashed overnight at 500°. Ashed samples were treated with hydrochloric acid, dried, and dissolved in 5% (v/v) hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, using inductively coupled plasma, with the emission of the standard solutions. The limits of quantitation of this method and Spex CertiPrep reference standards are listed in the table below.

Mineral	Lot Numbers	Concentration (µg/ml)	Limit of Quantitation (ppm)
Calcium	SC5179247, SC5179249	201.0, 1001	20.0
Copper	SC5179247, SC5179248	2.01, 10.04	0.50
Iron	SC5179247, SC5179250	9.99, 50.2	2.00
Magnesium	SC5179247, SC5179248	49.93, 250.0	20.0
Manganese	SC5179247, SC5179248	2.01, 10.06	0.30
Phosphorus	SC5179247, SC5179249	200.7, 1005	20.0
Potassium	SC5179247, SC5179249	199.9, 1007	100
Sodium	SC5179247, SC5179249	201.7, 1007	100
Zinc	SC5179247, SC5179248	9.92, 49.82	0.40

6.11 Moisture. The method used was based on AOAC International (2000) methods 926.08 and 925.09. Samples were dried in a vacuum oven at 100°C to a constant

weight. The moisture loss was determined and converted to percent moisture. The limit of quantitation of this method was 0.1% fw.

6.12 Neutral Detergent Fiber. The method used was based on AACC (1998) method 32.20 and an USDA Agriculture Handbook No. 379 (1970) method. Samples were placed in a fritted vessel and washed with a neutral boiling detergent solution to dissolve the protein, carbohydrate, enzyme, and ash. Fats and pigments were removed using an acetone wash. The hemicellulose, cellulose, and lignin fractions were collected on a frit and determined gravimetrically. The limit of quantitation of this method was 0.1% fw.

6.13 Niacin. The method used was based on AOAC International (2000) method 944.13. The grain sample was hydrolyzed with sulfuric acid and the pH was adjusted to remove interferences. The amount of niacin was turbidimetrically determined by comparing the growth response of the bacteria *Lactobacillus plantarum* in the samples versus the growth response in niacin standard. The limit of quantitation of this method was 0.3 µg/g fw. The reference standard was USP, niacin, 100%, lot number H2C121.

6.14 p-Coumaric and Ferulic Acids. The method was based on a literature method (Hagerman and Nicholson, 1982). The grain samples were extracted with methanol using ultrasonication, and the extracts were then hydrolyzed using 4N sodium hydroxide, buffered using acetic acid/sodium hydroxide, acidified with 3N hydrochloric acid, and filtered. The levels of p-coumaric and ferulic acids in the extracts were determined by RP-HPLC with UV quantitation. The reference standards were ACROS p-Hydroxycinnamic acid (p-coumaric acid), 97.9%, lot number A018661301 and ACROS 4-Hydroxy-3-methoxycinnamic acid (ferulic acid), 100%, lot number A014010401. The limit of quantitation for both analytes was calculated to be approximately 50.0 ppm using the following equation:

(conc. of lowest standard) × (vol) × (dil) / (sample weight) = quantitation limit (ppm)

6.15 Phytic Acid. The method used was based on two literature methods (Lehrfeld 1989, 1994). Grain samples were extracted using 0.5M HCl with ultrasonication. Purification and concentration was performed using a silica-based anion exchange (SAX) column. Sample analysis was conducted using a macroporous polymer HPLC column [PRP-1, 5µm (150 × 4.1 mm)] connected to a refractive index detector. The limit of quantitation of this method was approximately 0.1% fw. The reference standard was Aldrich phytic acid, dodecasodium salt hydrate, 95%, lot number 01913EC.

6.16 Protein. The method used was based on AOAC International (2000) methods 955.04 and 979.09 and two literature methods (Bradstreet, 1965; Kalthoff and Sandell, 1948). Protein and other nitrogenous compounds in the sample were

reduced to ammonia by digestion of the sample with sulfuric acid containing a mercury catalyst mixture. The acid digest was made alkaline, and the ammonia was distilled and titrated with a standard acid. The percent nitrogen was determined and converted to percent protein by multiplication with 6.25. The limit of quantitation of this method was 0.1% fw.

6.17 **Pyridoxine/Vitamin B_6.** The method used was based on AOAC International (2000) method 961.15. The grain sample was hydrolyzed with dilute sulfuric acid. The amount of pyridoxine was turbidimetrically determined by comparing the growth response of the yeast *Saccharomyces carlsbergensis* in the sample with the growth response in a pyridoxine standard. The limit of quantitation of this method was 0.07 µg/g fw. The reference standard was USP pyridoxine, 100%, lot number P.

6.18 **Raffinose.** This method was based on two literature methods (Mason and Slover, 1971; Brobst, 1972). The grain samples were extracted with deionized water and the extracts treated with an hydroxylamine hydrochloride solution in pyridine containing phenyl-β-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid and analyzed by gas chromatography using a flame ionization detector. The reference standard was Sigma, D(+)-Raffinose Pentahydrate Sigma Ultra, 99%, lot number 073K0938. The limit of quantitation of this method: The acceptable range for an 8/2.5 dilution was 0.05-0.9%.

6.19 **Riboflavin/Vitamin B_2.** The method used was based on AOAC International (2000) method 940.33. The grain sample was hydrolyzed with dilute HCl and pH adjusted to remove interferences. The amount of riboflavin was determined by comparing the growth response of the bacteria, *Lactobacillus casei*, in the sample hydrolysate with the bacterial growth response in varying amounts of riboflavin standard. The bacterial growth response was measured turbidimetrically. The limit of quantitation of this method was 0.2 µg/g fw. The reference standard was USP riboflavin, 100%, lot number N0C021.

6.20 **Thiamin/Vitamin B_1.** The method used was based on AOAC International (2000) methods 942.23, 953.17, and 957.17. The grain sample was autoclaved under weak acid conditions to extract the thiamin. The resulting solution was incubated with a buffered enzyme solution to release any bound thiamin. The solution was purified on an ion-exchange column. An aliquot was taken and reacted with potassium ferricyanide to convert thiamin to thiochrome. The thiochrome was extracted into isobutyl alcohol and read on a fluorometer against a known standard. The limit of quantitation of this method was 0.01 mg/100g fw. The reference standard was USP, thiamin, 100%, lot number O.

6.21 Total Dietary Fiber. The method used was based on AOAC International (2000) method 985.29. Duplicate grain samples were gelatinized with alpha-amylase and digested with enzymes to break down starch and protein. Ethanol was added to each sample to precipitate the soluble fiber. The samples were filtered and the residue was rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. Protein content was determined for one of the duplicates; ash content was determined for the other. The total dietary fiber in the sample was calculated using the protein and ash values. The limit of quantitation of this method was approximately 1.0% fw.

6.22 Vitamin E. The method used was based on three literature methods (Cort et al., 1983; McMurray et al., 1980; Speek et al., 1985). Grain samples were saponified to break down fat and release vitamin E. The saponified mixture was extracted with ethyl ether and quantitated directly by HPLC on a silica column. The limit of quantitation of this method was approximately 0.005 mg/g fw. The reference standard was USP alpha tocopherol, 100%, lot number M.

7.0 Control of Bias

The test, control, and reference substances from each respective plot within the Production Plan 04-01-50-02 field sites were produced under similar agronomic conditions. To control and/or minimize bias, the samples were analyzed in the order specified by a computer-generated randomized sample list. The Study Director generated the randomized sample list and forwarded it to Covance Laboratories Inc. prior to analysis.

8.0 Statistical Analysis

8.1 Data Processing

After compositional analyses were performed at Covance Laboratories Inc., data spreadsheets were sent to Monsanto Company. The data were reviewed, formatted, and sent to Certus International, Inc. for statistical analysis. A statistical sub-report was generated by Certus and sent to Monsanto Company (Reynolds et al., 2006).

The following formulas were used for re-expression of the data for statistical analysis:

Component	From (X)	To	Formula
Proximates (excluding moisture), Fiber, Raffinose, Phytic Acid	% FW	% DW	X/d
Furfural, p-Coumaric Acid, Ferulic Acid	ppm FW	ug/g DW	X/d
Calcium, Phosphorus, Magnesium, Potassium, Sodium	ppm FW	% DW	$(X/d) \times 10^{-4}$
Copper, Iron, Manganese, Zinc	ppm FW	mg/kg DW	X/d
Vitamin B1	mg/100g FW	mg/kg DW	10 (X/d)
Vitamin E	mg/g FW	mg/kg DW	10^3 (X/d)
Niacin, Folic Acid, Vitamin B2, Vitamin B6	ug/g FW	mg/kg DW	X/d
Amino Acids (AA)	mg/g FW	% DW	X/(10*d)
Fatty Acids (FA)	% FW	% Total FA	(100)Xj/Σ Xj, for each FA j
'd' is the fraction of the sample that is dry matter.			

The following 16 compositional analytes with >50% of observations below the LOQ of the assay were excluded from statistical analysis: sodium, furfural, raffinose, 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid.

The following additional seven observations for forage and grain tissue samples were below the LOQ: 16:1 palmitoleic acid (five values in grain); and vitamin E (two values in grain). To include a complete data set for these analytes in the statistical analysis, a value equal to half the quantitation limit was assigned for these seven data points.

The data was assessed for potential outliers using a studentized PRESS residuals calculation. Two outliers were identified in the data set: copper (Site 4, Test MON 89034, Rep 1), and iron (Site 1, Reference H8751, Rep 2). The identified copper and iron values were considered outliers and were removed from further analysis. The outlier test procedure was reapplied to all remaining copper and iron data to detect potential outliers that were masked in the first analysis. Only one iron value (Site 1, Test MON 89034, Rep 1) identified in the second analysis was considered an outlier and removed from further analysis.

8.2 Statistical Methodology

At the field sites, the test, control, and reference substances were grown in single plots randomly assigned within each of three replication blocks. The

compositional components for the test and control substances were statistically analyzed using a mixed model analysis of variance. The data from the five replicated sites were analyzed separately and as a combined data set. Individual replicated site analyses used the model:

$$Y_{ij} = U + T_i + B_j + e_{ij},$$

where Y_{ij} = unique individual observation, U = overall mean, T_i = hybrid effect, B_j = random block effect, and e_{ij} = residual error.

Combined site analyses used the model:

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk},$$

where Y_{ijk} = unique individual observation, U = overall mean, T_i = hybrid effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by hybrid interaction effect, and e_{ijk} = residual error. For each compositional component, the forage and grain from the test substance was compared to the conventional control.

A range of observed values from the reference substances was determined for each analytical component. Additionally, the reference substances data were used to develop population tolerance intervals. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of conventional references (George et al., 2004; Ridley et al., 2002c). Each tolerance interval estimate was based upon one observation per unique reference substance. Individual substances with multiple observations were summarized within sites to obtain a single estimate for inclusion in tolerance interval calculations. Because negative quantities are not possible, calculated negative lower tolerance bounds were set to zero. SAS® software was used to generate all summary statistics and perform all analyses (SAS Software Release 9.1, 2002-2003). Report tables present p-values from SAS® as either <0.001 or the actual value truncated to three decimal places.

9.0 Results and Discussion

The composition of forage and grain fromMON 89034 was analyzed and compared to conventional control corn and to the tolerance interval calculated from the conventional references. The compositional profile of each test, control, and reference substance was determined by evaluating 61 different analytes (nine in forage and 52 in grain). The compositional analyses of the test, control, and reference substances are found in the final

report of MSL # 20402 (Reynolds et al., 2006) and all of the data present in Tables 1-13 was obtained from the final report from MSL # 20402 (Reynolds et al., 2006). A statistical analysis summary was generated for each compositional analyte at each site and across all sites. A summary of the statistically significant differences ($p<0.05$) can be found in Table 1. Each test value that had a statistically significant difference from the comparator (i.e., $p<0.05$) was compared to the 99% tolerance interval generated from the reference substances in this study. For each component, least-square means, standard errors, and the range of observed values are presented for the test and control substances. In addition, mean differences between the test and control, standard errors for the mean differences, the range of observed differences, 95% confidence intervals of the differences and the significance probabilities are presented for each comparison in Tables 2-13. Reported literature and ILSI Crop Composition Database ranges for the analytical components found in corn forage and grain are in Table 14.

9.1 Composition Comparisons Between MON 89034 and Conventional Control

Statistical analyses for MON 89034 from the combined site showed statistically significant differences for three analytes. For two of these analytes, there were also statistically significant differences in more than one of the individual sites. For the remaining one analyte, there was a statistical difference in only one of the individual sites. Statistical analyses for MON 89034 from the five individual sites showed that 11 analytes were observed to be statistically different from the control in more than one of the individual sites and 33 analytes were observed to be statistically different from the control in only one of the individual sites (Table 1). Details of the statistical observations are as follows:

In the grain of the test substance, 20:0 arachidic acid was found to be statistically different from the control in the combined site. Statistical differences for 20:0 arachidic acid were also observed in three individual sites. Stearic acid (18:0) was found to be statistically different from the control in the combined site and in two individual sites. Phosphorus from the forage of the test substance was found to be statistically different from the control in the combined site and one of the five individual sites. Since statistical differences for 20:0 arachidic acid , 18:0 stearic acid, and phosphorus were observed in one to three of the five individual sites and the combined site, and the mean and range of values from the test substances were within the calculated 99% tolerance interval for the population of conventional reference substances, these differences were not considered to be biologically relevant.

Six comparisons (3 analytes) for MON 89034 were found to be statistically different from the control in more than one individual site and not in the combined site. Carbohydrate, iron, and copper values from the grain of MON 89034 were observed to be statistically different from the control at each of two individual field sites. Since the direction of change was not consistently observed across sites (for 2 of 3 analytes), and the mean and

range of values from the test substances were all within the calculated 99% tolerance interval for the population of conventional reference substances, these differences were not considered to be biologically relevant.

Thirty two comparisons for MON 89034 were found to be statistically different from the control in only one of the five individual sites and not in the combined site. For 31 of these comparisons, the mean and range of values for MON 89034 were within the calculated 99% tolerance interval for the population of conventional reference substances. The exceptions were calcium and methionine levels in grain grown in IA. The range of calcium and methionine values at this site fell within the values reported in the ILSI Crop Composition Database. Since these differences were observed at only one individual site, and all means and range of values from the test substance were within the range of values obtained from either the calculated 99% tolerance interval and/or the ILSI Crop Composition Database, these differences were not considered to be biologically relevant.

Based on statistical analyses of the combined site data and the individual site data, it is concluded that the forage and grain from MON 89034 are compositionally equivalent to conventional corn forage and grain.

10.0 Conclusions

In conclusion, data were generated and statistical analyses performed on forage and grain from MON 89034, a conventional control corn, and 15 conventional corn reference varieties. The statistical analyses showed that all of the 366 comparisons made between the test substance, MON 89034, and the conventional control corn substance, LH198 × LH172, were either: a) not significantly different, b) were significantly different ($p<0.05$) but the composition values for the test substances were within the calculated 99% tolerance interval for the population of conventional reference substances and not considered biologically relevant, or c) were significantly different ($p<0.05$) but the composition values for the test substances were within the range of values obtained from the ILSI Crop Composition Database and not considered biologically relevant. Thus, the forage and grain from MON 89034 are compositionally equivalent to conventional corn forage and grain.

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Table 1. Summary of Differences ($p<0.05$) for the Comparison of Maize Component Levels for Test (MON 89034) vs. the Conventional Control (LH198 x LH172) and Commercial Reference Substances

Analytical Component (Units)[1]	MON 89034 Mean	LH198 x LH172 Mean	Mean Difference (Test minus Comparator) % of LH198 x LH172	Mean Difference (Test minus Comparator) Signif. (p-Value)	MON 89034 (Range)	Commercial Tolerance Int.[2]
Statistical Differences Observed in Combined Site Analyses						
Mineral						
Forage Phosphorus (% DW)	0.25	0.21	19.24	0.010	(0.22 - 0.32)	[0.071,0.32]
Fatty Acid						
Grain 18:0 Stearic (% Total FA)	1.89	1.82	3.97	0.002	(1.79 - 2.03)	[0.86,2.98]
Grain 20:0 Arachidic (% Total FA)	0.39	0.38	3.43	<0.001	(0.36 - 0.42)	[0.23,0.54]
Statistical Differences Observed in More Than One Individual Site						
Proximate						
Site IA Grain Carbohydrates (% DW)	83.38	84.52	-1.34	0.008	(83.29 - 83.55)	[81.08,88.80]
Site OH Grain Carbohydrates (% DW)	84.26	83.80	0.55	0.009	(83.99 - 84.59)	[81.08,88.80]
Mineral						
Site IL-1 Grain Copper (mg/kg DW)	1.76	1.36	29.35	0.023	(1.51 - 2.21)	[0,4.20]
Site NE Grain Copper (mg/kg DW)	2.15	1.67	28.66	0.023	(1.92 - 2.38)	[0,4.20]
Site IL-1 Grain Iron (mg/kg DW)	20.86	19.48	7.11	0.048	(19.23 - 21.79)	[8.88,34.51]
Site OH Grain Iron (mg/kg DW)	21.37	25.74	-17.00	0.006	(20.59 - 21.76)	[8.88,34.51]
Fatty Acid						
Site IL-1 Grain 18:0 Stearic (% Total FA)	1.96	1.82	7.94	<0.001	(1.89 - 2.02)	[0.86,2.98]
Site IL-2 Grain 18:0 Stearic (% Total FA)	1.98	1.82	9.05	<0.001	(1.93 - 2.03)	[0.86,2.98]
Site IL-1 Grain 20:0 Arachidic (% Total FA)	0.41	0.39	5.23	0.007	(0.40 - 0.42)	[0.23,0.54]

Table 1. Summary of Differences (p<0.05) for the Comparison of Maize Component Levels for Test (MON 89034) vs. the Conventional Control (LH198 x LH172) and Commercial Reference Substances

Analytical Component (Units)[1]	MON 89034 Mean	LH198 x LH172 Mean	Mean Difference (Test minus Comparator) % of LH198 x LH172	Mean Difference (Test minus Comparator) Signif. (p-Value)	MON 89034 (Range)	Commercial Tolerance Int.[2]
Site IL-2 Grain 20:0 Arachidic (% Total FA)	0.39	0.37	6.83	0.021	(0.38 - 0.40)	[0.23,0.54]
Site OH Grain 20:0 Arachidic (% Total FA)	0.38	0.37	3.12	0.035	(0.38 - 0.39)	[0.23,0.54]
Statistical Differences Observed in One Individual Site Only						
Amino Acid						
Site IA Grain Alanine (% DW)	0.88	0.81	7.83	0.030	(0.87 - 0.88)	[0.48,1.08]
Site IA Grain Arginine (% DW)	0.51	0.46	10.83	0.005	(0.50 - 0.52)	[0.33,0.56]
Site IA Grain Aspartic acid (% DW)	0.77	0.71	8.66	0.003	(0.77 - 0.78)	[0.43,0.90]
Site IA Grain Cystine (% DW)	0.25	0.23	7.54	0.014	(0.24 - 0.26)	[0.18,0.27]
Site IA Grain Glutamic acid (% DW)	2.27	2.09	8.66	0.011	(2.26 - 2.28)	[1.25,2.75]
Site IA Grain Glycine (% DW)	0.41	0.38	6.94	0.020	(0.40 - 0.41)	[0.28,0.46]
Site IA Grain Histidine (% DW)	0.34	0.32	7.16	0.012	(0.34 - 0.34)	[0.22,0.38]
Site IA Grain Leucine (% DW)	1.49	1.37	8.96	0.032	(1.48 - 1.51)	[0.77,1.92]
Site IA Grain Lysine (% DW)	0.35	0.32	6.66	0.028	(0.33 - 0.36)	[0.20,0.40]
Site IA Grain Methionine (% DW)	0.25	0.23	11.20	0.003	(0.25 - 0.27)	[0.14,0.25]
Site IA Grain Phenylalanine (% DW)	0.58	0.53	9.45	0.028	(0.57 - 0.59)	[0.32,0.73]

Table 1. Summary of Differences ($p<0.05$) for the Comparison of Maize Component Levels for Test (MON 89034) vs. the Conventional Control (LH198 x LH172) and Commercial Reference Substances

Analytical Component (Units)[1]	MON 89034 Mean	LH198 x LH172 Mean	Mean Difference (Test minus Comparator) % of LH198 x LH172	Mean Difference (Test minus Comparator) Signif. (p-Value)	MON 89034 (Range)	Commercial Tolerance Int.[2]
Site IA Grain Proline (% DW)	1.05	0.98	7.29	0.028	(1.04 - 1.05)	[0.68,1.21]
Site IA Grain Serine (% DW)	0.60	0.56	8.28	0.004	(0.60 - 0.61)	[0.34,0.71]
Site IA Grain Threonine (% DW)	0.37	0.34	8.45	0.004	(0.37 - 0.37)	[0.24,0.41]
Proximate						
Site IA Grain Protein (% DW)	11.89	10.85	9.59	0.005	(11.73 - 11.98)	[7.54,13.13]
Site IL-1 Forage Moisture (% FW)	69.03	66.53	3.76	0.031	(68.50 - 69.40)	[57.62,86.45]
Site NE Forage Ash (% DW)	3.20	4.39	-27.12	0.021	(2.93 - 3.38)	[1.93,6.31]
Site NE Forage Carbohydrates (% DW)	88.16	84.98	3.74	0.004	(86.86 - 88.84)	[83.05,90.74]
Fiber						
Site NE Grain Neutral Detergent Fiber (% DW)	10.52	9.05	16.27	0.028	(10.43 - 10.69)	[5.93,13.63]
Site OH Forage Acid Detergent Fiber (% DW)	31.31	23.58	32.78	0.012	(26.92 - 34.93)	[16.76,43.76]
Site OH Forage Neutral Detergent Fiber (% DW)	43.21	37.87	14.11	0.027	(40.07 - 46.82)	[25.94,55.67]
Site IA Grain Tyrosine (% DW)	0.43	0.36	17.50	0.006	(0.42 - 0.43)	[0.17,0.52]

Table 1. Summary of Differences (p<0.05) for the Comparison of Maize Component Levels for Test (MON 89034) vs. the Conventional Control (LH198 x LH172) and Commercial Reference Substances

Analytical Component (Units)[1]	Test Mean	Comparator Mean	Mean Difference (Test minus Comparator) % of Comparator	Mean Difference (Test minus Comparator) Signif. (p-Value)	Test (Range)	Commercial Tolerance Int.[2]
Fatty Acid						
Site IA Grain 18:3 Linolenic (% Total FA)	1.21	1.34	-9.40	0.009	(1.20 - 1.23)	[0.63,1.77]
Site IL-1 Grain 16:1 Palmitoleic (% Total FA)	0.13	0.14	-6.87	0.012	(0.12 - 0.13)	[0,0.28]
Site IL-2 Grain 18:1 Oleic (% Total FA)	24.75	23.82	3.93	0.003	(24.14 - 25.25)	[7.51,46.46]
Site IL-2 Grain 18:2 Linoleic (% Total FA)	61.87	63.17	-2.07	0.001	(61.19 - 62.42)	[39.41,76.74]
Site NE Grain 20:1 Eicosenoic (% Total FA)	0.28	0.29	-1.50	0.030	(0.28 - 0.28)	[0.15,0.39]
Mineral						
Site IA Grain Calcium (% DW)	0.0064	0.0058	10.96	0.012	(0.0062 - 0.0066)	[0.0016,0.0059]
Site IA Grain Manganese (mg/kg DW)	8.34	6.99	19.32	0.017	(7.62 - 9.32)	[3.17,9.99]
Site IA Forage Calcium (% DW)	0.24	0.26	-8.77	0.033	(0.24 - 0.24)	[0.016,0.38]
Site NE Forage Phosphorus (% DW)	0.25	0.17	46.95	0.036	(0.23 - 0.28)	[0.071,0.32]
Vitamin						
Site IL-2 Grain Folic Acid (mg/kg DW)	0.37	0.32	13.81	<0.001	(0.35 - 0.38)	[0.012,0.69]
Secondary Metabolite						
Site OH Grain p-Coumaric Acid (µg/g DW)	218.38	185.63	17.64	0.032	(187.79 - 253.04)	[0,378.57]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; Combined Site = analyses of the combined data from each of the five replicated field trials.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 2. Statistical Summary of Site 1 Maize Forage Fiber, Calcium, Phosphorus, and Proximate Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fiber						
Acid Detergent Fiber (% DW)	33.94 ± 2.44 (32.27 - 35.85)	32.16 ± 2.44 (30.00 - 35.59)	1.78 ± 3.06 (0.27 - 2.79)	-5.28,8.83	0.577	(26.72 - 38.94) [16.76,43.76]
Neutral Detergent Fiber (% DW)	41.26 ± 0.80 (39.51 - 42.46)	42.26 ± 0.80 (40.23 - 43.96)	-1.00 ± 1.13 (-2.16 - -0.12)	-3.59,1.59	0.400	(33.70 - 46.74) [25.94,55.67]
Mineral						
Calcium (% DW)	0.24 ± 0.0065 (0.24 - 0.24)	0.26 ± 0.0065 (0.25 - 0.28)	-0.023 ± 0.0090 (-0.036 - -0.014)	-0.044,-0.0024	0.033	(0.11 - 0.29) [0.016,0.38]
Phosphorus (% DW)	0.24 ± 0.0048 (0.24 - 0.25)	0.24 ± 0.0048 (0.23 - 0.25)	0.0018 ± 0.0039 (-0.0019 - 0.0041)	-0.0072,0.011	0.654	(0.14 - 0.25) [0.071,0.32]
Proximate						
Ash (% DW)	4.21 ± 0.26 (3.32 - 4.67)	4.46 ± 0.26 (4.22 - 4.65)	-0.25 ± 0.36 (-1.19 - 0.42)	-1.08,0.59	0.515	(3.40 - 5.45) [1.93,6.31]
Carbohydrates (% DW)	85.50 ± 0.47 (85.20 - 85.68)	85.51 ± 0.47 (84.51 - 86.46)	-0.013 ± 0.60 (-0.85 - 1.18)	-1.40,1.38	0.983	(84.88 - 88.39) [83.05,90.74]
Moisture (% FW)	74.87 ± 0.44 (74.40 - 75.40)	73.83 ± 0.44 (72.70 - 74.40)	1.03 ± 0.51 (0.40 - 1.70)	-0.13,2.20	0.075	(64.90 - 77.40) [57.62,86.45]
Protein (% DW)	8.90 ± 0.16 (8.85 - 8.98)	8.49 ± 0.16 (8.24 - 8.87)	0.41 ± 0.21 (0.12 - 0.63)	-0.085,0.91	0.092	(6.58 - 8.82) [4.78,10.38]
Total Fat (% DW)	1.39 ± 0.60 (0.89 - 2.13)	1.54 ± 0.60 (0.92 - 2.75)	-0.15 ± 0.66 (-0.61 - 0.23)	-1.66,1.36	0.823	(0.58 - 3.11) [0,4.54]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 3. Statistical Summary of Site 1 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Alanine (% DW)	0.88 ± 0.017 (0.87 - 0.88)	0.81 ± 0.017 (0.79 - 0.84)	0.064 ± 0.024 (0.037 - 0.089)	0.0078,0.12	0.030	(0.67 - 0.96) [0.48,1.08]
Arginine (% DW)	0.51 ± 0.0095 (0.50 - 0.52)	0.46 ± 0.0095 (0.46 - 0.47)	0.050 ± 0.013 (0.036 - 0.062)	0.020,0.081	0.005	(0.37 - 0.49) [0.33,0.56]
Aspartic acid (% DW)	0.77 ± 0.011 (0.77 - 0.78)	0.71 ± 0.011 (0.70 - 0.73)	0.061 ± 0.015 (0.038 - 0.078)	0.026,0.097	0.003	(0.57 - 0.77) [0.43,0.90]
Cystine (% DW)	0.25 ± 0.0039 (0.24 - 0.26)	0.23 ± 0.0039 (0.23 - 0.23)	0.017 ± 0.0056 (0.011 - 0.023)	0.0045,0.030	0.014	(0.20 - 0.24) [0.18,0.27]
Glutamic acid (% DW)	2.27 ± 0.039 (2.26 - 2.28)	2.09 ± 0.039 (2.03 - 2.16)	0.18 ± 0.055 (0.12 - 0.24)	0.054,0.31	0.011	(1.71 - 2.41) [1.25,2.75]
Glycine (% DW)	0.41 ± 0.0065 (0.40 - 0.41)	0.38 ± 0.0065 (0.37 - 0.39)	0.026 ± 0.0091 (0.012 - 0.035)	0.0052,0.047	0.020	(0.32 - 0.40) [0.28,0.46]
Histidine (% DW)	0.34 ± 0.0057 (0.34 - 0.34)	0.32 ± 0.0057 (0.31 - 0.32)	0.023 ± 0.0081 (0.015 - 0.030)	0.0041,0.041	0.022	(0.26 - 0.33) [0.22,0.38]
Isoleucine (% DW)	0.39 ± 0.0099 (0.39 - 0.40)	0.37 ± 0.0099 (0.36 - 0.38)	0.025 ± 0.014 (0.016 - 0.041)	-0.0075,0.057	0.114	(0.32 - 0.45) [0.23,0.51]

Table 3. Statistical Summary of Site 1 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Leucine (% DW)	1.49 ± 0.034 (1.48 - 1.51)	1.37 ± 0.034 (1.33 - 1.41)	0.12 ± 0.047 (0.098 - 0.16)	0.013,0.23	0.032	(1.14 - 1.68) [0.77,1.92]
Lysine (% DW)	0.35 ± 0.0062 (0.33 - 0.36)	0.32 ± 0.0062 (0.32 - 0.33)	0.022 ± 0.0081 (0.0042 - 0.033)	0.0029,0.040	0.028	(0.24 - 0.34) [0.20,0.40]
Methionine (% DW)	0.25 ± 0.0043 (0.25 - 0.27)	0.23 ± 0.0043 (0.22 - 0.24)	0.026 ± 0.0061 (0.024 - 0.028)	0.012,0.040	0.003	(0.17 - 0.22) [0.14,0.25]
Phenylalanine (% DW)	0.58 ± 0.013 (0.57 - 0.59)	0.53 ± 0.013 (0.52 - 0.54)	0.050 ± 0.019 (0.041 - 0.067)	0.0066,0.094	0.028	(0.45 - 0.65) [0.32,0.73]
Proline (% DW)	1.05 ± 0.019 (1.04 - 1.05)	0.98 ± 0.019 (0.95 - 1.01)	0.071 ± 0.027 (0.041 - 0.10)	0.0097,0.13	0.028	(0.83 - 1.11) [0.68,1.21]
Serine (% DW)	0.60 ± 0.0085 (0.60 - 0.61)	0.56 ± 0.0085 (0.55 - 0.57)	0.046 ± 0.012 (0.034 - 0.058)	0.019,0.074	0.004	(0.45 - 0.62) [0.34,0.71]
Threonine (% DW)	0.37 ± 0.0051 (0.37 - 0.37)	0.34 ± 0.0051 (0.33 - 0.36)	0.029 ± 0.0072 (0.016 - 0.039)	0.012,0.046	0.004	(0.29 - 0.37) [0.24,0.41]
Tryptophan (% DW)	0.062 ± 0.0011 (0.061 - 0.063)	0.061 ± 0.0011 (0.058 - 0.063)	0.0016 ± 0.0015 (-0.0015 - 0.0050)	-0.0018,0.0051	0.311	(0.043 - 0.059) [0.032,0.072]

Table 3. Statistical Summary of Site 1 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.43 ± 0.012 (0.42 - 0.43)	0.36 ± 0.012 (0.35 - 0.37)	0.063 ± 0.018 (0.052 - 0.072)	0.023,0.10	0.006	(0.25 - 0.40) [0.17,0.52]
Valine (% DW)	0.53 ± 0.012 (0.53 - 0.54)	0.50 ± 0.012 (0.48 - 0.51)	0.035 ± 0.016 (0.020 - 0.055)	-0.0026,0.073	0.063	(0.42 - 0.55) [0.35,0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	9.21 ± 0.043 (9.12 - 9.31)	9.23 ± 0.043 (9.15 - 9.34)	-0.023 ± 0.060 (-0.14 - 0.16)	-0.16,0.11	0.706	(9.10 - 12.55) [6.12,15.67]
16:1 Palmitoleic (% Total FA)	0.11 ± 0.0081 (0.11 - 0.11)	0.11 ± 0.0081 (0.11 - 0.12)	-0.0017 ± 0.011 (-0.0034 - 0.00039)	-0.028,0.025	0.889	(0.050 - 0.19) [0,0.28]
18:0 Stearic (% Total FA)	1.80 ± 0.016 (1.79 - 1.83)	1.81 ± 0.016 (1.77 - 1.85)	-0.0070 ± 0.023 (-0.055 - 0.063)	-0.061,0.047	0.772	(1.57 - 2.45) [0.86,2.98]
18:1 Oleic (% Total FA)	25.08 ± 0.12 (24.87 - 25.36)	24.75 ± 0.12 (24.55 - 24.92)	0.34 ± 0.17 (0.099 - 0.81)	-0.067,0.73	0.083	(21.17 - 35.33) [7.51,46.46]
18:2 Linoleic (% Total FA)	61.79 ± 0.12 (61.56 - 62.00)	61.98 ± 0.12 (61.74 - 62.18)	-0.19 ± 0.17 (-0.45 - 0.25)	-0.58,0.20	0.298	(50.33 - 63.59) [39.41,76.74]
18:3 Linolenic (% Total FA)	1.21 ± 0.026 (1.20 - 1.23)	1.34 ± 0.026 (1.25 - 1.43)	-0.13 ± 0.037 (-0.23 - -0.022)	-0.21,-0.040	0.009	(0.93 - 1.52) [0.63,1.77]

Table 3. Statistical Summary of Site 1 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.37 ± 0.0057 (0.36 - 0.39)	0.37 ± 0.0057 (0.36 - 0.38)	0.0036 ± 0.0081 (-0.019 - 0.032)	-0.015,0.022	0.670	(0.32 - 0.47) [0.23,0.54]
20:1 Eicosenoic (% Total FA)	0.27 ± 0.0050 (0.26 - 0.28)	0.27 ± 0.0050 (0.25 - 0.28)	0.0018 ± 0.0064 (-0.014 - 0.011)	-0.013,0.016	0.784	(0.23 - 0.32) [0.15,0.39]
22:0 Behenic (% Total FA)	0.15 ± 0.0035 (0.14 - 0.16)	0.14 ± 0.0035 (0.14 - 0.15)	0.0030 ± 0.0049 (-0.0071 - 0.017)	-0.0083,0.014	0.562	(0.12 - 0.19) [0.081,0.23]
Fiber						
Acid Detergent Fiber (% DW)	5.39 ± 0.30 (4.76 - 5.80)	5.19 ± 0.30 (4.76 - 5.68)	0.20 ± 0.42 (-0.92 - 1.04)	-0.76,1.17	0.641	(4.11 - 6.33) [2.77,7.56]
Neutral Detergent Fiber (% DW)	11.31 ± 0.28 (10.78 - 12.08)	10.68 ± 0.28 (9.93 - 11.22)	0.63 ± 0.38 (-0.44 - 1.20)	-0.25,1.52	0.136	(8.20 - 11.30) [5.93,13.63]
Total Dietary Fiber (% DW)	15.37 ± 0.46 (14.70 - 16.28)	14.22 ± 0.46 (13.62 - 15.25)	1.15 ± 0.65 (-0.55 - 2.50)	-0.34,2.65	0.112	(12.99 - 18.03) [9.20,20.27]
Mineral						
Calcium (% DW)	0.0064 ± 0.00014 (0.0062 - 0.0066)	0.0058 ± 0.00014 (0.0056 - 0.0059)	0.00063 ± 0.00020 (0.00043 - 0.00090)	0.00018,0.0011	0.012	(0.0031 - 0.0049) [0.0016,0.0059]
Copper (mg/kg DW)	1.89 ± 0.69 (1.86 - 1.95)	2.82 ± 0.69 (1.68 - 4.54)	-0.92 ± 0.98 (-2.59 - 0.18)	-3.17,1.33	0.372	(1.15 - 3.56) [0,4.20]

Table 3. Statistical Summary of Site 1 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Mineral						
Iron (mg/kg DW)	23.64 ± 1.22 (23.02 - 24.06)	25.49 ± 1.00 (24.07 - 27.02)	-1.96 ± 1.58 (-4.01 - -1.33)	-5.69,1.77	0.254	(18.04 - 29.22) [8.88,34.51]
Magnesium (% DW)	0.13 ± 0.0021 (0.13 - 0.13)	0.12 ± 0.0021 (0.12 - 0.13)	0.0036 ± 0.0030 (-0.0012 - 0.0087)	-0.0034,0.011	0.269	(0.099 - 0.14) [0.075,0.17]
Manganese (mg/kg DW)	8.34 ± 0.34 (7.62 - 9.32)	6.99 ± 0.34 (6.84 - 7.17)	1.35 ± 0.45 (0.78 - 2.36)	0.30,2.40	0.017	(5.56 - 8.64) [3.17,9.99]
Phosphorus (% DW)	0.34 ± 0.0049 (0.34 - 0.35)	0.34 ± 0.0049 (0.33 - 0.35)	0.0050 ± 0.0070 (-0.0068 - 0.015)	-0.011,0.021	0.496	(0.25 - 0.37) [0.18,0.45]
Potassium (% DW)	0.37 ± 0.0060 (0.36 - 0.38)	0.37 ± 0.0060 (0.36 - 0.38)	-0.00086 ± 0.0084 (-0.010 - 0.0055)	-0.020,0.019	0.921	(0.32 - 0.40) [0.26,0.46]
Zinc (mg/kg DW)	26.50 ± 0.58 (25.91 - 26.89)	25.46 ± 0.58 (24.53 - 26.04)	1.04 ± 0.82 (0.098 - 2.18)	-0.85,2.93	0.239	(16.72 - 34.04) [7.16,38.55]
Proximate						
Ash (% DW)	1.48 ± 0.056 (1.38 - 1.56)	1.44 ± 0.056 (1.35 - 1.49)	0.043 ± 0.063 (0.021 - 0.076)	-0.10,0.19	0.513	(1.12 - 1.62) [0.74,1.96]
Carbohydrates (% DW)	83.38 ± 0.23 (83.29 - 83.55)	84.52 ± 0.23 (84.28 - 84.74)	-1.13 ± 0.32 (-1.42 - -0.98)	-1.88,-0.39	0.008	(82.91 - 86.78) [81.08,88.80]

Table 3. Statistical Summary of Site 1 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Proximate						
Moisture (% FW)	8.06 ± 0.096 (7.89 - 8.16)	8.09 ± 0.096 (7.86 - 8.21)	-0.030 ± 0.14 (-0.080 - 0.030)	-0.34,0.28	0.830	(7.60 - 15.30) [0.45,19.52]
Protein (% DW)	11.89 ± 0.19 (11.73 - 11.98)	10.85 ± 0.19 (10.70 - 11.00)	1.04 ± 0.27 (0.87 - 1.28)	0.41,1.67	0.005	(9.33 - 11.82) [7.54,13.13]
Total Fat (% DW)	3.24 ± 0.047 (3.16 - 3.33)	3.19 ± 0.047 (3.13 - 3.24)	0.050 ± 0.067 (-0.0014 - 0.12)	-0.10,0.20	0.479	(2.66 - 3.71) [2.20,4.55]
Vitamin						
Folic Acid (mg/kg DW)	0.41 ± 0.048 (0.39 - 0.43)	0.51 ± 0.048 (0.49 - 0.53)	-0.10 ± 0.059 (-0.11 - -0.098)	-0.24,0.034	0.121	(0.13 - 0.45) [0.012,0.69]
Niacin (mg/kg DW)	28.14 ± 0.74 (26.27 - 30.05)	29.01 ± 0.74 (27.34 - 29.85)	-0.88 ± 1.04 (-3.57 - 2.71)	-3.28,1.52	0.424	(16.17 - 29.19) [6.97,37.83]
Vitamin B1 (mg/kg DW)	3.05 ± 0.16 (2.39 - 3.38)	3.23 ± 0.16 (3.05 - 3.36)	-0.18 ± 0.21 (-0.66 - 0.11)	-0.67,0.30	0.411	(2.19 - 5.60) [0.37,6.35]
Vitamin B2 (mg/kg DW)	1.45 ± 0.11 (1.37 - 1.52)	1.45 ± 0.11 (1.36 - 1.51)	-0.0076 ± 0.15 (-0.12 - 0.16)	-0.36,0.34	0.961	(1.34 - 1.91) [0.91,2.30]
Vitamin B6 (mg/kg DW)	6.74 ± 0.11 (6.49 - 6.99)	6.73 ± 0.11 (6.67 - 6.80)	0.016 ± 0.16 (-0.31 - 0.32)	-0.35,0.38	0.922	(5.08 - 7.47) [3.12,9.30]

Table 3. Statistical Summary of Site 1 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Vitamin						
Vitamin E (mg/kg DW)	6.33 ± 0.71 (5.73 - 6.70)	4.87 ± 0.71 (2.72 - 6.00)	1.46 ± 1.01 (-0.27 - 3.83)	-0.86,3.78	0.185	(2.71 - 13.94) [0,20.49]
Antinutrient						
Phytic Acid (% DW)	0.78 ± 0.039 (0.77 - 0.80)	0.75 ± 0.039 (0.70 - 0.83)	0.028 ± 0.055 (-0.037 - 0.073)	-0.099,0.15	0.629	(0.50 - 0.94) [0.21,1.22]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	2458.21 ± 53.43 (2366.74 - 2525.31)	2571.23 ± 53.43 (2472.77 - 2669.85)	-113.03 ± 75.57 (-303.11 - 9.81)	-287.29,61.23	0.173	(1412.68 - 2297.36) [1136.69,2806.24]
p-Coumaric Acid (µg/g DW)	172.95 ± 6.96 (166.11-177.48)	172.63 ± 6.96 (167.76-176.90)	0.32 ± 9.63 (-10.80 – 9.76)	-21.89,22.53	0.974	(99.30 – 285.75) [0,378.57]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 4. Statistical Summary of Site 2 Maize Forage Fiber, Calcium, Phosphorus, and Proximate Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fiber						
Acid Detergent Fiber (% DW)	25.53 ± 1.39 (24.18 - 26.38)	27.40 ± 1.39 (24.53 - 32.26)	-1.87 ± 1.97 (-6.22 - 1.85)	-6.42,2.68	0.371	(26.72 - 38.94) [16.76,43.76]
Neutral Detergent Fiber (% DW)	36.81 ± 1.89 (33.99 - 39.94)	36.96 ± 1.89 (35.78 - 37.65)	-0.15 ± 2.67 (-3.47 - 4.16)	-6.32,6.02	0.956	(33.70 - 46.74) [25.94,55.67]
Mineral						
Calcium (% DW)	0.17 ± 0.0093 (0.16 - 0.18)	0.17 ± 0.0093 (0.15 - 0.19)	0.0044 ± 0.012 (-0.017 - 0.017)	-0.023,0.031	0.716	(0.11 - 0.29) [0.016,0.38]
Phosphorus (% DW)	0.24 ± 0.014 (0.23 - 0.24)	0.20 ± 0.014 (0.18 - 0.24)	0.041 ± 0.019 (0.0048 - 0.064)	-0.0036,0.085	0.066	(0.14 - 0.25) [0.071,0.32]
Proximate						
Ash (% DW)	3.55 ± 0.30 (3.30 - 3.93)	3.57 ± 0.30 (2.96 - 4.24)	-0.015 ± 0.43 (-0.94 - 0.97)	-1.00,0.97	0.972	(3.40 - 5.45) [1.93,6.31]
Carbohydrates (% DW)	86.92 ± 0.87 (84.98 - 88.60)	88.22 ± 0.87 (85.87 - 89.57)	-1.30 ± 1.23 (-4.23 - 2.73)	-4.13,1.53	0.320	(84.88 - 88.39) [83.05,90.74]
Moisture (% FW)	69.03 ± 0.68 (68.50 - 69.40)	66.53 ± 0.68 (65.90 - 67.70)	2.50 ± 0.96 (1.70 - 3.30)	0.29,4.71	0.031	(64.90 - 77.40) [57.62,86.45]
Protein (% DW)	7.53 ± 0.39 (6.95 - 8.41)	6.63 ± 0.39 (6.06 - 7.52)	0.90 ± 0.56 (-0.30 - 2.35)	-0.38,2.18	0.144	(6.58 - 8.82) [4.78,10.38]
Total Fat (% DW)	2.00 ± 0.45 (0.88 - 3.17)	1.58 ± 0.45 (1.16 - 2.37)	0.42 ± 0.64 (-1.49 - 1.95)	-1.05,1.88	0.528	(0.58 - 3.11) [0,4.54]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 5. Statistical Summary of Site 2 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Alanine (% DW)	0.70 ± 0.027 (0.66 - 0.76)	0.71 ± 0.027 (0.67 - 0.78)	-0.0092 ± 0.033 (-0.012 - -0.0057)	-0.084,0.066	0.785	(0.67 - 0.96) [0.48,1.08]
Arginine (% DW)	0.45 ± 0.011 (0.43 - 0.47)	0.45 ± 0.011 (0.44 - 0.47)	0.0049 ± 0.015 (-0.0068 - 0.015)	-0.031,0.040	0.760	(0.37 - 0.49) [0.33,0.56]
Aspartic acid (% DW)	0.62 ± 0.019 (0.60 - 0.66)	0.62 ± 0.019 (0.60 - 0.67)	0.0013 ± 0.023 (-0.0033 - 0.0070)	-0.052,0.054	0.956	(0.57 - 0.77) [0.43,0.90]
Cystine (% DW)	0.22 ± 0.0058 (0.22 - 0.23)	0.22 ± 0.0058 (0.21 - 0.23)	0.0044 ± 0.0069 (-0.0022 - 0.014)	-0.011,0.020	0.538	(0.20 - 0.24) [0.18,0.27]
Glutamic acid (% DW)	1.78 ± 0.073 (1.69 - 1.95)	1.81 ± 0.073 (1.70 - 2.00)	-0.025 ± 0.085 (-0.051 - -0.0056)	-0.22,0.17	0.779	(1.71 - 2.41) [1.25,2.75]
Glycine (% DW)	0.37 ± 0.0084 (0.36 - 0.39)	0.37 ± 0.0084 (0.36 - 0.38)	0.0048 ± 0.011 (0.0023 - 0.0062)	-0.020,0.030	0.665	(0.32 - 0.40) [0.28,0.46]
Histidine (% DW)	0.29 ± 0.0082 (0.27 - 0.31)	0.29 ± 0.0082 (0.28 - 0.30)	0.0011 ± 0.0087 (-0.012 - 0.011)	-0.019,0.021	0.898	(0.26 - 0.33) [0.22,0.38]
Isoleucine (% DW)	0.32 ± 0.014 (0.30 - 0.36)	0.32 ± 0.014 (0.30 - 0.36)	0.00013 ± 0.017 (-0.0070 - 0.010)	-0.038,0.038	0.993	(0.32 - 0.45) [0.23,0.51]

Table 5. Statistical Summary of Site 2 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Leucine (% DW)	1.15 ± 0.055 (1.09 - 1.27)	1.17 ± 0.055 (1.08 - 1.33)	-0.017 ± 0.064 (-0.052 - 0.0032)	-0.16,0.13	0.803	(1.14 - 1.68) [0.77,1.92]
Lysine (% DW)	0.32 ± 0.0090 (0.29 - 0.33)	0.30 ± 0.0090 (0.29 - 0.31)	0.017 ± 0.012 (0.0028 - 0.031)	-0.0093,0.044	0.170	(0.24 - 0.34) [0.20,0.40]
Methionine (% DW)	0.22 ± 0.0056 (0.21 - 0.23)	0.22 ± 0.0056 (0.20 - 0.23)	0.0033 ± 0.0079 (-0.0066 - 0.014)	-0.015,0.021	0.682	(0.17 - 0.22) [0.14,0.25]
Phenylalanine (% DW)	0.46 ± 0.019 (0.44 - 0.50)	0.46 ± 0.019 (0.43 - 0.52)	-0.0020 ± 0.022 (-0.018 - 0.0087)	-0.052,0.048	0.929	(0.45 - 0.65) [0.32,0.73]
Proline (% DW)	0.89 ± 0.031 (0.84 - 0.96)	0.88 ± 0.031 (0.84 - 0.95)	0.0040 ± 0.037 (-0.0064 - 0.011)	-0.082,0.090	0.917	(0.83 - 1.11) [0.68,1.21]
Serine (% DW)	0.48 ± 0.014 (0.46 - 0.50)	0.49 ± 0.014 (0.46 - 0.52)	-0.0096 ± 0.018 (-0.021 - 0.0088)	-0.051,0.032	0.607	(0.45 - 0.62) [0.34,0.71]
Threonine (% DW)	0.31 ± 0.0081 (0.30 - 0.33)	0.31 ± 0.0081 (0.29 - 0.32)	0.0067 ± 0.0091 (-0.00043 - 0.011)	-0.014,0.028	0.484	(0.29 - 0.37) [0.24,0.41]
Tryptophan (% DW)	0.054 ± 0.0024 (0.053 - 0.056)	0.055 ± 0.0024 (0.052 - 0.061)	-0.00092 ± 0.0026 (-0.0055 - 0.0014)	-0.0070,0.0051	0.735	(0.043 - 0.059) [0.032,0.072]

Table 5. Statistical Summary of Site 2 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference(Test minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.34 ± 0.012 (0.33 - 0.36)	0.35 ± 0.012 (0.32 - 0.38)	-0.0023 ± 0.013 (-0.024 - 0.015)	-0.033,0.028	0.863	(0.25 - 0.40) [0.17,0.52]
Valine (% DW)	0.45 ± 0.016 (0.42 - 0.50)	0.45 ± 0.016 (0.43 - 0.49)	0.0016 ± 0.020 (-0.011 - 0.0084)	-0.045,0.048	0.938	(0.42 - 0.55) [0.35,0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	9.21 ± 0.065 (9.17 - 9.24)	9.08 ± 0.065 (8.91 - 9.23)	0.12 ± 0.089 (-0.013 - 0.33)	-0.080,0.33	0.199	(9.10 - 12.55) [6.12,15.67]
16:1 Palmitoleic (% Total FA)	0.13 ± 0.0022 (0.12 - 0.13)	0.14 ± 0.0022 (0.13 - 0.14)	-0.0053 ± 0.0029 (-0.011 - -0.0071)	-0.016,-0.0027	0.012	(0.050 - 0.19) [0,0.28]
18:0 Stearic (% Total FA)	1.96 ± 0.027 (1.89 - 2.02)	1.82 ± 0.027 (1.76 - 1.85)	0.14 ± 0.024 (0.12 - 0.18)	0.088,0.20	<0.001	(1.57 - 2.45) [0.86,2.98]
18:1 Oleic (% Total FA)	25.30 ± 0.29 (25.03 - 25.68)	25.78 ± 0.29 (25.34 - 26.66)	-0.48 ± 0.32 (-0.98 - -0.15)	-1.21,0.25	0.168	(21.17 - 35.33) [7.51,46.46]
18:2 Linoleic (% Total FA)	61.34 ± 0.22 (61.02 - 61.54)	61.14 ± 0.22 (60.51 - 61.53)	0.20 ± 0.27 (-0.051 - 0.51)	-0.42,0.82	0.471	(50.33 - 63.59) [39.41,76.74]
18:3 Linolenic (% Total FA)	1.22 ± 0.011 (1.21 - 1.23)	1.21 ± 0.011 (1.19 - 1.23)	0.014 ± 0.016 (-0.014 - 0.036)	-0.022,0.051	0.390	(0.93 - 1.52) [0.63,1.77]

Table 5. Statistical Summary of Site 2 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.41 ± 0.0055 (0.40 - 0.42)	0.39 ± 0.0055 (0.38 - 0.40)	0.020 ± 0.0057 (0.017 - 0.023)	0.0073,0.034	0.007	(0.32 - 0.47) [0.23,0.54]
20:1 Eicosenoic (% Total FA)	0.29 ± 0.0052 (0.28 - 0.29)	0.29 ± 0.0052 (0.29 - 0.29)	-0.0035 ± 0.0073 (-0.0040 - -0.0029)	-0.020,0.013	0.644	(0.23 - 0.32) [0.15,0.39]
22:0 Behenic (% Total FA)	0.15 ± 0.0042 (0.14 - 0.15)	0.16 ± 0.0042 (0.15 - 0.16)	-0.013 ± 0.0057 (-0.019 - -0.0013)	-0.026,0	0.050	(0.12 - 0.19) [0.081,0.23]
Fiber						
Acid Detergent Fiber (% DW)	4.96 ± 0.51 (3.82 - 6.05)	5.55 ± 0.51 (4.37 - 7.00)	-0.59 ± 0.71 (-3.18 - 1.69)	-2.24,1.06	0.435	(4.11 - 6.33) [2.77,7.56]
Neutral Detergent Fiber (% DW)	10.00 ± 0.53 (9.83 - 10.11)	10.50 ± 0.53 (9.48 - 11.22)	-0.50 ± 0.75 (-1.16 - 0.34)	-2.24,1.24	0.524	(8.20 - 11.30) [5.93,13.63]
Total Dietary Fiber (% DW)	14.49 ± 0.70 (13.39 - 15.06)	14.93 ± 0.70 (13.17 - 15.84)	-0.43 ± 0.99 (-2.45 - 1.88)	-2.71,1.84	0.671	(12.99 - 18.03) [9.20,20.27]
Mineral						
Calcium (% DW)	0.0048 ± 0.00018 (0.0046 - 0.0049)	0.0048 ± 0.00018 (0.0046 - 0.0050)	-0.00004 ± 0.00021 (-0.00006 - -0.00003)	-0.00054,0.00045	0.845	(0.0031 - 0.0049) [0.0016,0.0059]
Copper (mg/kg DW)	1.76 ± 0.11 (1.51 - 2.21)	1.36 ± 0.11 (1.26 - 1.43)	0.40 ± 0.14 (0.16 - 0.78)	0.071,0.73	0.023	(1.15 - 3.56) [0,4.20]

Table 5. Statistical Summary of Site 2 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Mineral						
Iron (mg/kg DW)	20.86 ± 0.42 (19.23 - 20.79)	19.48 ± 0.42 (19.03 - 19.71)	1.38 ± 0.60 (-0.47 - 2.75)	0.012,2.76	0.048	(18.04 - 29.22) [8.88,34.51]
Magnesium (% DW)	0.12 ± 0.0022 (0.11 - 0.12)	0.11 ± 0.0022 (0.11 - 0.12)	0.0044 ± 0.0029 (-0.0066 - 0.011)	-0.0023,0.011	0.170	(0.099 - 0.14) [0.075,0.17]
Manganese (mg/kg DW)	6.54 ± 0.17 (6.11 - 6.78)	6.19 ± 0.17 (6.03 - 6.47)	0.35 ± 0.24 (-0.36 - 0.71)	-0.20,0.89	0.180	(5.56 - 8.64) [3.17,9.99]
Phosphorus (% DW)	0.33 ± 0.0062 (0.31 - 0.35)	0.33 ± 0.0062 (0.32 - 0.35)	0.0070 ± 0.0088 (-0.021 - 0.026)	-0.013,0.027	0.449	(0.25 - 0.37) [0.18,0.45]
Potassium (% DW)	0.38 ± 0.0084 (0.37 - 0.40)	0.37 ± 0.0084 (0.36 - 0.40)	0.011 ± 0.011 (-0.030 - 0.035)	-0.014,0.035	0.345	(0.32 - 0.40) [0.26,0.46]
Zinc (mg/kg DW)	20.50 ± 0.52 (18.91 - 22.12)	19.26 ± 0.52 (18.81 - 20.03)	1.24 ± 0.74 (-1.13 - 3.19)	-0.46,2.94	0.131	(16.72 - 34.04) [7.16,38.55]
Proximate						
Ash (% DW)	1.40 ± 0.038 (1.35 - 1.47)	1.43 ± 0.038 (1.34 - 1.48)	-0.025 ± 0.053 (-0.11 - 0.046)	-0.15,0.097	0.648	(1.12 - 1.62) [0.74,1.96]
Carbohydrates (% DW)	85.63 ± 0.27 (84.90 - 86.11)	85.67 ± 0.27 (84.94 - 86.22)	-0.044 ± 0.27 (-0.11 - 0.012)	-0.68,0.59	0.875	(82.91 - 86.78) [81.08,88.80]

Table 5. Statistical Summary of Site 2 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Proximate						
Moisture (% FW)	8.46 ± 0.22 (8.15 - 9.02)	8.43 ± 0.22 (8.06 - 9.08)	0.030 ± 0.31 (-0.93 - 0.87)	-0.69,0.75	0.925	(7.60 - 15.30) [0.45,19.52]
Protein (% DW)	9.67 ± 0.29 (9.14 - 10.35)	9.67 ± 0.29 (9.22 - 10.50)	0.0034 ± 0.23 (-0.15 - 0.30)	-0.53,0.53	0.988	(9.33 - 11.82) [7.54,13.13]
Total Fat (% DW)	3.30 ± 0.093 (3.26 - 3.36)	3.23 ± 0.093 (3.09 - 3.45)	0.066 ± 0.11 (-0.20 - 0.21)	-0.18,0.31	0.553	(2.66 - 3.71) [2.20,4.55]
Vitamin						
Folic Acid (mg/kg DW)	0.30 ± 0.017 (0.27 - 0.33)	0.33 ± 0.017 (0.30 - 0.36)	-0.030 ± 0.024 (-0.058 - 0.0038)	-0.086,0.026	0.249	(0.13 - 0.45) [0.012,0.69]
Niacin (mg/kg DW)	32.34 ± 1.44 (30.61 - 34.84)	32.70 ± 1.44 (31.03 - 35.75)	-0.36 ± 2.04 (-4.17 - 3.81)	-5.06,4.34	0.865	(16.17 - 29.19) [6.97,37.83]
Vitamin B1 (mg/kg DW)	3.17 ± 0.18 (3.05 - 3.27)	2.84 ± 0.18 (2.39 - 3.16)	0.33 ± 0.26 (0.030 - 0.66)	-0.27,0.93	0.242	(2.19 - 5.60) [0.37,6.35]
Vitamin B2 (mg/kg DW)	1.46 ± 0.069 (1.35 - 1.65)	1.53 ± 0.069 (1.45 - 1.61)	-0.072 ± 0.098 (-0.22 - 0.20)	-0.30,0.15	0.484	(1.34 - 1.91) [0.91,2.30]
Vitamin B6 (mg/kg DW)	6.49 ± 0.14 (6.27 - 6.64)	6.53 ± 0.14 (6.45 - 6.63)	-0.042 ± 0.16 (-0.37 - 0.20)	-0.41,0.33	0.800	(5.08 - 7.47) [3.12,9.30]

Table 5. Statistical Summary of Site 2 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Vitamin						
Vitamin E (mg/kg DW)	7.16 ± 0.26 (6.47 - 7.91)	6.96 ± 0.26 (6.65 - 7.40)	0.20 ± 0.35 (-0.93 - 1.08)	-0.62,1.01	0.591	(2.71 - 13.94) [0,20.49]
Antinutrient						
Phytic Acid (% DW)	0.86 ± 0.041 (0.83 - 0.87)	0.78 ± 0.041 (0.69 - 0.85)	0.081 ± 0.059 (-0.013 - 0.18)	-0.055,0.22	0.206	(0.50 - 0.94) [0.21,1.22]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	2057.02 ± 106.60 (1923.50 - 2298.73)	2184.45 ± 106.60 (2033.94 - 2265.73)	-127.43 ± 150.76 (-330.17 - 264.79)	-475.07,220.22	0.422	(1412.68 - 2297.36) [1136.69,2806.24]
p-Coumaric Acid (µg/g DW)	196.97 ± 7.65 (185.76 – 214.62)	195.82 ± 7.65 (188.08- 210.13)	1.15 ± 10.81 (-24.37 – 25.37)	-23.78,26.08	0.917	(99.30 – 285.75) [0,378.57]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 6. Statistical Summary of Site 3 Maize Forage Fiber, Calcium, Phosphorus, and Proximate Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference(Test minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fiber						
Acid Detergent Fiber (% DW)	29.76 ± 1.78 (26.56 - 33.83)	28.84 ± 1.78 (25.00 - 31.08)	0.92 ± 2.27 (-3.89 - 3.89)	-4.32,6.15	0.696	(26.72 - 38.94) [16.76,43.76]
Neutral Detergent Fiber (% DW)	39.23 ± 2.36 (34.41 - 42.19)	37.97 ± 2.36 (35.41 - 42.21)	1.26 ± 2.13 (-1.11 - 5.89)	-3.66,6.19	0.570	(33.70 - 46.74) [25.94,55.67]
Mineral						
Calcium (% DW)	0.22 ± 0.016 (0.22 - 0.22)	0.21 ± 0.016 (0.19 - 0.23)	0.011 ± 0.022 (-0.014 - 0.034)	-0.038,0.061	0.615	(0.11 - 0.29) [0.016,0.38]
Phosphorus (% DW)	0.22 ± 0.0097 (0.22 - 0.23)	0.20 ± 0.0097 (0.19 - 0.21)	0.026 ± 0.014 (0.016 - 0.033)	-0.0052,0.058	0.090	(0.14 - 0.25) [0.071,0.32]
Proximate						
Ash (% DW)	4.36 ± 0.24 (3.99 - 4.57)	4.13 ± 0.24 (3.78 - 4.47)	0.23 ± 0.34 (-0.48 - 0.74)	-0.55,1.01	0.522	(3.40 - 5.45) [1.93,6.31]
Carbohydrates (% DW)	86.08 ± 0.54 (84.93 - 86.69)	86.94 ± 0.54 (86.69 - 87.13)	-0.85 ± 0.76 (-2.05 - -0.060)	-2.60,0.89	0.292	(84.88 - 88.39) [83.05,90.74]
Moisture (% FW)	74.03 ± 0.82 (73.00 - 74.70)	75.13 ± 0.82 (73.00 - 76.80)	-1.10 ± 0.99 (-2.40 - 0)	-3.38,1.18	0.297	(64.90 - 77.40) [57.62,86.45]
Protein (% DW)	8.15 ± 0.36 (7.59 - 8.75)	7.85 ± 0.36 (7.54 - 8.07)	0.30 ± 0.51 (-0.33 - 1.21)	-0.88,1.48	0.574	(6.58 - 8.82) [4.78,10.38]
Total Fat (% DW)	1.41 ± 0.19 (1.20 - 1.75)	1.08 ± 0.19 (0.77 - 1.31)	0.32 ± 0.27 (0.037 - 0.51)	-0.30,0.95	0.262	(0.58 - 3.11) [0,4.54]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 7. Statistical Summary of Site 3 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Alanine (% DW)	0.69 ± 0.020 (0.67 - 0.71)	0.71 ± 0.020 (0.67 - 0.74)	-0.028 ± 0.025 (-0.070 - 0.0025)	-0.085,0.028	0.282	(0.67 - 0.96) [0.48,1.08]
Arginine (% DW)	0.47 ± 0.012 (0.44 - 0.49)	0.45 ± 0.012 (0.41 - 0.47)	0.017 ± 0.017 (-0.023 - 0.056)	-0.022,0.057	0.342	(0.37 - 0.49) [0.33,0.56]
Aspartic acid (% DW)	0.63 ± 0.014 (0.60 - 0.66)	0.64 ± 0.014 (0.61 - 0.66)	-0.0031 ± 0.019 (-0.040 - 0.028)	-0.047,0.041	0.875	(0.57 - 0.77) [0.43,0.90]
Cystine (% DW)	0.21 ± 0.0055 (0.20 - 0.21)	0.22 ± 0.0055 (0.21 - 0.23)	-0.0091 ± 0.0078 (-0.022 - 0.0052)	-0.027,0.0088	0.274	(0.20 - 0.24) [0.18,0.27]
Glutamic acid (% DW)	1.77 ± 0.053 (1.71 - 1.82)	1.84 ± 0.053 (1.73 - 1.90)	-0.077 ± 0.068 (-0.19 - 0.040)	-0.23,0.079	0.286	(1.71 - 2.41) [1.25,2.75]
Glycine (% DW)	0.38 ± 0.0066 (0.36 - 0.39)	0.37 ± 0.0066 (0.36 - 0.38)	0.0089 ± 0.0094 (-0.0046 - 0.017)	-0.013,0.031	0.372	(0.32 - 0.40) [0.28,0.46]
Histidine (% DW)	0.29 ± 0.0061 (0.28 - 0.30)	0.29 ± 0.0061 (0.28 - 0.30)	0.0017 ± 0.0083 (-0.0026 - 0.0066)	-0.017,0.021	0.841	(0.26 - 0.33) [0.22,0.38]
Isoleucine (% DW)	0.34 ± 0.0097 (0.32 - 0.34)	0.34 ± 0.0097 (0.33 - 0.36)	-0.0078 ± 0.014 (-0.018 - 0.012)	-0.039,0.024	0.587	(0.32 - 0.45) [0.23,0.51]

Table 7. Statistical Summary of Site 3 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Leucine (% DW)	1.15 ± 0.041 (1.12 - 1.18)	1.21 ± 0.041 (1.13 - 1.26)	-0.064 ± 0.051 (-0.13 - 0.015)	-0.18,0.053	0.243	(1.14 - 1.68) [0.77,1.92]
Lysine (% DW)	0.33 ± 0.0098 (0.31 - 0.35)	0.31 ± 0.0098 (0.29 - 0.32)	0.021 ± 0.014 (0.017 - 0.023)	-0.011,0.053	0.163	(0.24 - 0.34) [0.20,0.40]
Methionine (% DW)	0.20 ± 0.0056 (0.20 - 0.20)	0.21 ± 0.0056 (0.20 - 0.22)	-0.012 ± 0.0071 (-0.017 - -0.0048)	-0.029,0.0039	0.117	(0.17 - 0.22) [0.14,0.25]
Phenylalanine (% DW)	0.46 ± 0.015 (0.45 - 0.48)	0.48 ± 0.015 (0.45 - 0.50)	-0.016 ± 0.019 (-0.039 - 0.016)	-0.060,0.027	0.414	(0.45 - 0.65) [0.32,0.73]
Proline (% DW)	0.88 ± 0.020 (0.87 - 0.91)	0.88 ± 0.020 (0.83 - 0.91)	0.00035 ± 0.025 (-0.035 - 0.035)	-0.057,0.057	0.989	(0.83 - 1.11) [0.68,1.21]
Serine (% DW)	0.46 ± 0.011 (0.45 - 0.49)	0.48 ± 0.011 (0.46 - 0.50)	-0.019 ± 0.014 (-0.053 - 0)	-0.051,0.014	0.227	(0.45 - 0.62) [0.34,0.71]
Threonine (% DW)	0.31 ± 0.0085 (0.30 - 0.32)	0.31 ± 0.0085 (0.30 - 0.32)	0.00064 ± 0.010 (-0.015 - 0.013)	-0.023,0.024	0.951	(0.29 - 0.37) [0.24,0.41]
Tryptophan (% DW)	0.050 ± 0.0025 (0.048 - 0.052)	0.050 ± 0.0025 (0.045 - 0.054)	-0.00022 ± 0.0032 (-0.0051 - 0.0068)	-0.0075,0.0071	0.946	(0.043 - 0.059) [0.032,0.072]

Table 7. Statistical Summary of Site 3 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.36 ± 0.024 (0.35 - 0.36)	0.32 ± 0.024 (0.24 - 0.38)	0.034 ± 0.021 (-0.014 - 0.11)	-0.015,0.082	0.150	(0.25 - 0.40) [0.17,0.52]
Valine (% DW)	0.46 ± 0.011 (0.45 - 0.48)	0.46 ± 0.011 (0.45 - 0.48)	-0.00051 ± 0.016 (-0.019 - 0.021)	-0.036,0.035	0.974	(0.42 - 0.55) [0.35,0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	9.29 ± 0.069 (9.12 - 9.46)	9.10 ± 0.069 (9.06 - 9.16)	0.19 ± 0.092 (0.050 - 0.30)	-0.019,0.41	0.068	(9.10 - 12.55) [6.12,15.67]
16:1 Palmitoleic (% Total FA)	0.12 ± 0.0018 (0.12 - 0.13)	0.13 ± 0.0018 (0.12 - 0.13)	-0.0005 ± 0.0026 (-0.012 - 0.0013)	-0.011,0.00046	0.066	(0.050 - 0.19) [0,0.28]
18:0 Stearic (% Total FA)	1.98 ± 0.024 (1.93 - 2.03)	1.82 ± 0.024 (1.79 - 1.85)	0.16 ± 0.032 (0.13 - 0.18)	0.090,0.24	<0.001	(1.57 - 2.45) [0.86,2.98]
18:1 Oleic (% Total FA)	24.75 ± 0.18 (24.14 - 25.25)	23.82 ± 0.18 (23.62 - 24.11)	0.94 ± 0.23 (0.52 - 1.15)	0.40,1.47	0.003	(21.17 - 35.33) [7.51,46.46]
18:2 Linoleic (% Total FA)	61.87 ± 0.23 (61.19 - 62.42)	63.17 ± 0.23 (62.80 - 63.41)	-1.30 ± 0.28 (-1.62 - -1.00)	-1.95,-0.66	0.001	(50.33 - 63.59) [39.41,76.74]
18:3 Linolenic (% Total FA)	1.17 ± 0.014 (1.12 - 1.22)	1.18 ± 0.014 (1.15 - 1.21)	-0.013 ± 0.018 (-0.033 - 0.024)	-0.054,0.029	0.505	(0.93 - 1.52) [0.63,1.77]

Table 7. Statistical Summary of Site 3 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.39 ± 0.0063 (0.38 - 0.40)	0.37 ± 0.0063 (0.36 - 0.37)	0.025 ± 0.0088 (0.015 - 0.032)	0.0047,0.045	0.021	(0.32 - 0.47) [0.23,0.54]
20:1 Eicosenoic (% Total FA)	0.28 ± 0.0034 (0.27 - 0.28)	0.27 ± 0.0034 (0.27 - 0.28)	0.0022 ± 0.0046 (-0.0050 - 0.0073)	-0.0083,0.013	0.644	(0.23 - 0.32) [0.15,0.39]
22:0 Behenic (% Total FA)	0.14 ± 0.0053 (0.13 - 0.15)	0.14 ± 0.0053 (0.14 - 0.15)	0.0017 ± 0.0075 (-0.012 - 0.011)	-0.016,0.019	0.830	(0.12 - 0.19) [0.081,0.23]
Fiber						
Acid Detergent Fiber (% DW)	5.53 ± 0.37 (4.53 - 6.10)	4.95 ± 0.37 (4.54 - 5.46)	0.58 ± 0.50 (-0.32 - 1.42)	-0.57,1.73	0.277	(4.11 - 6.33) [2.77,7.56]
Neutral Detergent Fiber (% DW)	8.98 ± 0.84 (8.59 - 9.49)	9.63 ± 0.84 (8.48 - 11.75)	-0.65 ± 1.19 (-2.26 - 0.20)	-3.40,2.10	0.601	(8.20 - 11.30) [5.93,13.63]
Total Dietary Fiber (% DW)	15.11 ± 0.90 (14.02 - 17.02)	14.75 ± 0.90 (12.82 - 17.62)	0.36 ± 1.25 (-3.61 - 4.20)	-2.53,3.24	0.782	(12.99 - 18.03) [9.20,20.27]
Mineral						
Calcium (% DW)	0.0040 ± 0.00009 (0.0038 - 0.0042)	0.0041 ± 0.00009 (0.0040 - 0.0044)	-0.00014 ± 0.00011 (-0.00027 - -0.00001)	-0.00039,0.00010	0.216	(0.0031 - 0.0049) [0.0016,0.0059]
Copper (mg/kg DW)	1.61 ± 0.57 (1.50 - 1.72)	1.81 ± 0.57 (1.61 - 1.93)	-0.19 ± 0.81 (-0.43 - 0.10)	-2.06,1.68	0.818	(1.15 - 3.56) [0,4.20]

Table 7. Statistical Summary of Site 3 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Mineral						
Iron (mg/kg DW)	19.62 ± 0.27 (19.23 - 19.91)	20.28 ± 0.27 (19.34 - 20.89)	-0.66 ± 0.39 (-1.66 - 0.38)	-1.55,0.23	0.126	(18.04 - 29.22) [8.88,34.51]
Magnesium (% DW)	0.11 ± 0.0026 (0.11 - 0.12)	0.11 ± 0.0026 (0.11 - 0.12)	-0.00043 ± 0.0027 (-0.0055 - 0.0051)	-0.0067,0.0058	0.879	(0.099 - 0.14) [0.075,0.17]
Manganese (mg/kg DW)	5.65 ± 0.14 (5.43 - 5.80)	5.74 ± 0.14 (5.57 - 5.85)	-0.084 ± 0.13 (-0.36 - 0.15)	-0.39,0.22	0.540	(5.56 - 8.64) [3.17,9.99]
Phosphorus (% DW)	0.32 ± 0.0077 (0.31 - 0.32)	0.32 ± 0.0077 (0.30 - 0.34)	-0.0021 ± 0.0097 (-0.015 - 0.013)	-0.020,0.025	0.831	(0.25 - 0.37) [0.18,0.45]
Potassium (% DW)	0.37 ± 0.0075 (0.37 - 0.38)	0.36 ± 0.0075 (0.35 - 0.38)	0.011 ± 0.011 (-0.0066 - 0.022)	-0.014,0.035	0.342	(0.32 - 0.40) [0.26,0.46]
Zinc (mg/kg DW)	19.73 ± 0.33 (19.35 - 20.00)	20.13 ± 0.33 (19.39 - 20.67)	-0.40 ± 0.44 (-0.66 - -0.040)	-1.42,0.61	0.387	(16.72 - 34.04) [7.16,38.55]
Proximate						
Ash (% DW)	1.38 ± 0.092 (1.35 - 1.44)	1.31 ± 0.092 (1.28 - 1.35)	0.069 ± 0.13 (0.0050 - 0.13)	-0.23,0.37	0.607	(1.12 - 1.62) [0.74,1.96]
Carbohydrates (% DW)	85.86 ± 0.31 (85.08 - 86.52)	85.68 ± 0.31 (85.53 - 85.84)	0.18 ± 0.44 (-0.44 - 0.84)	-0.85,1.20	0.699	(82.91 - 86.78) [81.08,88.80]

Table 7. Statistical Summary of Site 3 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Proximate						
Moisture (% FW)	9.79 ± 0.16 (9.51 - 10.10)	9.60 ± 0.16 (9.51 - 9.77)	0.19 ± 0.23 (0 - 0.33)	-0.34,0.72	0.441	(7.60 - 15.30) [0.45,19.52]
Protein (% DW)	9.11 ± 0.27 (8.54 - 9.67)	9.58 ± 0.27 (9.38 - 9.80)	-0.47 ± 0.38 (-1.26 - 0.29)	-1.34,0.41	0.252	(9.33 - 11.82) [7.54,13.13]
Total Fat (% DW)	3.65 ± 0.099 (3.50 - 3.89)	3.43 ± 0.099 (3.22 - 3.75)	0.22 ± 0.13 (0.15 - 0.29)	-0.079,0.52	0.127	(2.66 - 3.71) [2.20,4.55]
Vitamin						
Folic Acid (mg/kg DW)	0.37 ± 0.0059 (0.35 - 0.38)	0.32 ± 0.0059 (0.32 - 0.33)	0.045 ± 0.0084 (0.028 - 0.057)	0.025,0.064	<0.001	(0.13 - 0.45) [0.012,0.69]
Niacin (mg/kg DW)	31.66 ± 1.26 (27.14 - 34.70)	30.16 ± 1.26 (29.06 - 31.59)	1.49 ± 1.78 (-4.44 - 5.64)	-2.62,5.61	0.426	(16.17 - 29.19) [6.97,37.83]
Vitamin B1 (mg/kg DW)	2.81 ± 0.23 (2.66 - 2.98)	2.65 ± 0.23 (2.55 - 2.76)	0.15 ± 0.33 (-0.10 - 0.33)	-0.63,0.94	0.657	(2.19 - 5.60) [0.37,6.35]
Vitamin B2 (mg/kg DW)	1.43 ± 0.060 (1.38 - 1.51)	1.28 ± 0.060 (1.20 - 1.39)	0.15 ± 0.085 (0.0051 - 0.27)	-0.046,0.35	0.115	(1.34 - 1.91) [0.91,2.30]
Vitamin B6 (mg/kg DW)	5.82 ± 0.17 (5.65 - 6.13)	5.89 ± 0.17 (5.67 - 6.07)	-0.070 ± 0.25 (-0.38 - 0.46)	-0.64,0.50	0.784	(5.08 - 7.47) [3.12,9.30]

Table 7. Statistical Summary of Site 3 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Vitamin						
Vitamin E (mg/kg DW)	6.66 ± 0.18 (6.43 - 6.98)	6.38 ± 0.18 (6.05 - 6.82)	0.28 ± 0.19 (-0.39 - 0.71)	-0.16,0.72	0.182	(2.71 - 13.94) [0,20.49]
Antinutrient						
Phytic Acid (% DW)	0.66 ± 0.046 (0.56 - 0.79)	0.67 ± 0.046 (0.65 - 0.70)	-0.0089 ± 0.066 (-0.14 - 0.13)	-0.16,0.14	0.896	(0.50 - 0.94) [0.21,1.22]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1995.62 ± 93.94 (1790.25 - 2124.58)	1961.58 ± 93.94 (1878.66 - 2122.02)	34.04 ± 103.91 (-88.41 - 240.51)	-205.59,273.66	0.751	(1412.68 - 2297.36) [1136.69,2806.24]
p-Coumaric Acid (µg/g DW)	186.61 ± 8.59 (172.39 – 195.01)	188.43 ± 8.59 (171.29 – 198.38)	-1.82 ± 9.99 (-5.95 – 1.11)	-24.86,21.22	0.860	(99.30 – 285.75) [0,378.57]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 8. Statistical Summary of Site 4 Maize Forage Fiber, Calcium, Phosphorus, and Proximate Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fiber						
Acid Detergent Fiber (% DW)	24.19 ± 1.74 (22.60 - 27.08)	24.29 ± 1.74 (19.93 - 26.90)	-0.11 ± 2.47 (-4.03 - 7.15)	-5.80,5.58	0.965	(26.72 - 38.94) [16.76,43.76]
Neutral Detergent Fiber (% DW)	37.93 ± 2.41 (35.64 - 39.24)	32.96 ± 2.41 (31.44 - 34.62)	4.97 ± 3.41 (2.83 - 7.47)	-2.89,12.84	0.182	(33.70 - 46.74) [25.94,55.67]
Mineral						
Calcium (% DW)	0.17 ± 0.0084 (0.16 - 0.18)	0.16 ± 0.0084 (0.13 - 0.17)	0.010 ± 0.012 (-0.017 - 0.049)	-0.017,0.038	0.415	(0.11 - 0.29) [0.016,0.38]
Phosphorus (% DW)	0.25 ± 0.023 (0.23 - 0.28)	0.17 ± 0.023 (0.15 - 0.21)	0.080 ± 0.032 (0.024 - 0.13)	0.0064,0.15	0.036	(0.14 - 0.25) [0.071,0.32]
Proximate						
Ash (% DW)	3.20 ± 0.29 (2.93 - 3.38)	4.39 ± 0.29 (3.30 - 5.10)	-1.19 ± 0.42 (-1.72 - -0.37)	-2.15,-0.23	0.021	(3.40 - 5.45) [1.93,6.31]
Carbohydrates (% DW)	88.16 ± 0.65 (86.86 - 88.84)	84.98 ± 0.65 (84.36 - 85.29)	3.18 ± 0.82 (1.57 - 4.41)	1.29,5.07	0.004	(84.88 - 88.39) [83.05,90.74]
Moisture (% FW)	71.73 ± 1.01 (69.70 - 74.30)	72.23 ± 1.01 (70.10 - 74.70)	-0.50 ± 1.31 (-3.50 - 4.20)	-3.51,2.51	0.711	(64.90 - 77.40) [57.62,86.45]
Protein (% DW)	7.03 ± 0.38 (6.34 - 7.52)	8.02 ± 0.38 (7.63 - 8.66)	-0.99 ± 0.54 (-2.32 - -0.23)	-2.23,0.25	0.104	(6.58 - 8.82) [4.78,10.38]
Total Fat (% DW)	1.61 ± 0.43 (0.63 - 2.33)	2.62 ± 0.43 (2.18 - 2.91)	-1.00 ± 0.46 (-2.28 - 0.15)	-2.05,0.049	0.059	(0.58 - 3.11) [0,4.54]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 9. Statistical Summary of Site 4 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Alanine (% DW)	0.73 ± 0.023 (0.64 - 0.78)	0.77 ± 0.023 (0.76 - 0.78)	-0.043 ± 0.033 (-0.13 - 0.0071)	-0.12,0.032	0.223	(0.67 - 0.96) [0.48,1.08]
Arginine (% DW)	0.44 ± 0.018 (0.38 - 0.48)	0.47 ± 0.018 (0.46 - 0.48)	-0.028 ± 0.025 (-0.090 - 0.010)	-0.085,0.029	0.289	(0.37 - 0.49) [0.33,0.56]
Aspartic acid (% DW)	0.63 ± 0.020 (0.56 - 0.67)	0.67 ± 0.020 (0.66 - 0.68)	-0.041 ± 0.028 (-0.11 - -0.0031)	-0.11,0.024	0.182	(0.57 - 0.77) [0.43,0.90]
Cystine (% DW)	0.24 ± 0.0042 (0.23 - 0.25)	0.24 ± 0.0042 (0.23 - 0.25)	0.00021 ± 0.0060 (-0.012 - 0.012)	-0.014,0.014	0.973	(0.20 - 0.24) [0.18,0.27]
Glutamic acid (% DW)	1.86 ± 0.062 (1.63 - 2.01)	1.99 ± 0.062 (1.96 - 2.00)	-0.12 ± 0.088 (-0.33 - 0.011)	-0.33,0.080	0.200	(1.71 - 2.41) [1.25,2.75]
Glycine (% DW)	0.36 ± 0.012 (0.32 - 0.39)	0.38 ± 0.012 (0.38 - 0.39)	-0.023 ± 0.017 (-0.067 - 0.00013)	-0.061,0.016	0.212	(0.32 - 0.40) [0.28,0.46]
Histidine (% DW)	0.29 ± 0.0094 (0.25 - 0.32)	0.31 ± 0.0094 (0.30 - 0.31)	-0.016 ± 0.013 (-0.050 - 0.0033)	-0.046,0.015	0.266	(0.26 - 0.33) [0.22,0.38]
Isoleucine (% DW)	0.34 ± 0.012 (0.30 - 0.37)	0.36 ± 0.012 (0.36 - 0.36)	-0.019 ± 0.017 (-0.056 - 0.0027)	-0.057,0.020	0.294	(0.32 - 0.45) [0.23,0.51]

Table 9. Statistical Summary of Site 4 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Leucine (% DW)	1.25 ± 0.040 (1.09 - 1.33)	1.33 ± 0.040 (1.30 - 1.35)	-0.080 ± 0.057 (-0.21 - -0.015)	-0.21,0.051	0.195	(1.14 - 1.68) [0.77,1.92]
Lysine (% DW)	0.30 ± 0.013 (0.26 - 0.32)	0.31 ± 0.013 (0.31 - 0.32)	-0.018 ± 0.018 (-0.056 - 0.0089)	-0.060,0.024	0.349	(0.24 - 0.34) [0.20,0.40]
Methionine (% DW)	0.23 ± 0.0038 (0.22 - 0.24)	0.23 ± 0.0038 (0.22 - 0.24)	-0.0036 ± 0.0054 (-0.014 - 0.0072)	-0.016,0.0090	0.528	(0.17 - 0.22) [0.14,0.25]
Phenylalanine (% DW)	0.49 ± 0.016 (0.43 - 0.52)	0.52 ± 0.016 (0.51 - 0.53)	-0.030 ± 0.023 (-0.080 - -0.0037)	-0.083,0.023	0.231	(0.45 - 0.65) [0.32,0.73]
Proline (% DW)	0.90 ± 0.028 (0.79 - 0.97)	0.94 ± 0.028 (0.93 - 0.96)	-0.043 ± 0.040 (-0.15 - 0.012)	-0.14,0.049	0.314	(0.83 - 1.11) [0.68,1.21]
Serine (% DW)	0.49 ± 0.014 (0.44 - 0.54)	0.52 ± 0.014 (0.52 - 0.53)	-0.031 ± 0.020 (-0.087 - 0.0053)	-0.077,0.015	0.160	(0.45 - 0.62) [0.34,0.71]
Threonine (% DW)	0.31 ± 0.011 (0.27 - 0.34)	0.34 ± 0.011 (0.33 - 0.35)	-0.024 ± 0.015 (-0.052 - -0.00037)	-0.058,0.011	0.155	(0.29 - 0.37) [0.24,0.41]
Tryptophan (% DW)	0.054 ± 0.0020 (0.051 - 0.056)	0.055 ± 0.0020 (0.052 - 0.057)	-0.00057 ± 0.0029 (-0.0032 - 0.0035)	-0.0072,0.0060	0.846	(0.043 - 0.059) [0.032,0.072]

Table 9. Statistical Summary of Site 4 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.36 ± 0.011 (0.32 - 0.38)	0.39 ± 0.011 (0.38 - 0.40)	-0.029 ± 0.016 (-0.066 - -0.0014)	-0.065,0.0073	0.103	(0.25 - 0.40) [0.17,0.52]
Valine (% DW)	0.46 ± 0.016 (0.40 - 0.49)	0.48 ± 0.016 (0.48 - 0.49)	-0.026 ± 0.022 (-0.084 - 0.0093)	-0.078,0.026	0.277	(0.42 - 0.55) [0.35,0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	9.26 ± 0.050 (9.14 - 9.35)	9.23 ± 0.050 (9.19 - 9.29)	0.037 ± 0.071 (-0.051 - 0.14)	-0.13,0.20	0.619	(9.10 - 12.55) [6.12,15.67]
16:1 Palmitoleic (% Total FA)	0.14 ± 0.010 (0.13 - 0.14)	0.14 ± 0.010 (0.13 - 0.14)	0.00086 ± 0.015 (-0.00036 - 0.0019)	-0.033,0.035	0.954	(0.050 - 0.19) [0,0.28]
18:0 Stearic (% Total FA)	1.88 ± 0.019 (1.86 - 1.89)	1.85 ± 0.019 (1.82 - 1.87)	0.029 ± 0.027 (0.021 - 0.034)	-0.033,0.090	0.318	(1.57 - 2.45) [0.86,2.98]
18:1 Oleic (% Total FA)	25.60 ± 0.23 (25.42 - 25.75)	25.59 ± 0.23 (24.96 - 25.98)	0.0060 ± 0.28 (-0.41 - 0.66)	-0.83,0.64	0.983	(21.17 - 35.33) [7.51,46.46]
18:2 Linoleic (% Total FA)	61.12 ± 0.24 (60.85 - 61.27)	61.19 ± 0.24 (60.77 - 61.91)	-0.071 ± 0.32 (-0.64 - 0.35)	-0.82,0.67	0.830	(50.33 - 63.59) [39.41,76.74]
18:3 Linolenic (% Total FA)	1.18 ± 0.020 (1.17 - 1.18)	1.19 ± 0.020 (1.16 - 1.20)	-0.0096 ± 0.022 (-0.031 - 0.019)	-0.059,0.040	0.669	(0.93 - 1.52) [0.63,1.77]

Table 9. Statistical Summary of Site 4 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.38 ± 0.0020 (0.38 - 0.39)	0.38 ± 0.0020 (0.38 - 0.39)	0.0039 ± 0.0023 (0.00019 - 0.0078)	-0.0014,0.0091	0.130	(0.32 - 0.47) [0.23,0.54]
20:1 Eicosenoic (% Total FA)	0.28 ± 0.0012 (0.28 - 0.28)	0.29 ± 0.0012 (0.28 - 0.29)	-0.0043 ± 0.0016 (-0.0071 - -0.0018)	-0.0081,-0.00052	0.030	(0.23 - 0.32) [0.15,0.39]
22:0 Behenic (% Total FA)	0.17 ± 0.016 (0.15 - 0.18)	0.16 ± 0.016 (0.13 - 0.18)	0.0092 ± 0.018 (-0.0098 - 0.022)	-0.031,0.050	0.612	(0.12 - 0.19) [0.081,0.23]
Fiber						
Acid Detergent Fiber (% DW)	5.55 ± 0.41 (5.06 - 5.94)	5.41 ± 0.41 (5.28 - 5.49)	0.14 ± 0.58 (-0.43 - 0.49)	-1.19,1.47	0.817	(4.11 - 6.33) [2.77,7.56]
Neutral Detergent Fiber (% DW)	10.52 ± 0.42 (10.43 - 10.69)	9.05 ± 0.42 (8.64 - 9.38)	1.47 ± 0.55 (1.07 - 2.05)	0.20,2.75	0.028	(8.20 - 11.30) [5.93,13.63]
Total Dietary Fiber (% DW)	16.51 ± 0.66 (16.27 - 16.76)	15.63 ± 0.66 (15.07 - 16.69)	0.88 ± 0.93 (-0.17 - 1.63)	-1.26,3.03	0.368	(12.99 - 18.03) [9.20,20.27]
Mineral						
Calcium (% DW)	0.0050 ± 0.00016 (0.0048 - 0.0054)	0.0050 ± 0.00016 (0.0047 - 0.0051)	0.00008 ± 0.00023 (-0.00024 - 0.00064)	-0.00044,0.00061	0.722	(0.0031 - 0.0049) [0.0016,0.0059]
Copper (mg/kg DW)	2.15 ± 0.13 (1.92 - 2.38)	1.67 ± 0.11 (1.54 - 1.75)	0.48 ± 0.17 (0.38 - 0.63)	0.086,0.87	0.023	(1.15 - 3.56) [0,4.20]

Table 9. Statistical Summary of Site 4 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Mineral						
Iron (mg/kg DW)	21.47 ± 0.93 (19.45 - 23.23)	20.02 ± 0.93 (19.33 - 20.71)	1.45 ± 1.32 (-0.98 - 5.90)	-1.60,4.49	0.304	(18.04 - 29.22) [8.88,34.51]
Magnesium (% DW)	0.10 ± 0.0038 (0.10 - 0.11)	0.11 ± 0.0038 (0.11 - 0.12)	-0.0075 ± 0.0047 (-0.018 - 0.0036)	-0.018,0.0032	0.145	(0.099 - 0.14) [0.075,0.17]
Manganese (mg/kg DW)	6.85 ± 0.33 (6.45 - 7.45)	7.11 ± 0.33 (6.66 - 8.00)	-0.26 ± 0.47 (-1.54 - 0.78)	-1.35,0.82	0.590	(5.56 - 8.64) [3.17,9.99]
Phosphorus (% DW)	0.29 ± 0.010 (0.27 - 0.31)	0.30 ± 0.010 (0.29 - 0.31)	-0.011 ± 0.012 (-0.038 - 0.016)	-0.038,0.016	0.357	(0.25 - 0.37) [0.18,0.45]
Potassium (% DW)	0.34 ± 0.0082 (0.32 - 0.37)	0.35 ± 0.0082 (0.34 - 0.35)	-0.0086 ± 0.0087 (-0.029 - 0.014)	-0.029,0.012	0.352	(0.32 - 0.40) [0.26,0.46]
Zinc (mg/kg DW)	21.39 ± 0.80 (20.07 - 23.74)	22.46 ± 0.80 (21.75 - 23.44)	-1.07 ± 1.14 (-3.37 - 1.53)	-3.69,1.54	0.371	(16.72 - 34.04) [7.16,38.55]
Proximate						
Ash (% DW)	1.34 ± 0.042 (1.25 - 1.38)	1.35 ± 0.042 (1.30 - 1.40)	-0.013 ± 0.059 (-0.043 - 0.030)	-0.15,0.12	0.826	(1.12 - 1.62) [0.74,1.96]
Carbohydrates (% DW)	85.11 ± 0.22 (84.99 - 85.29)	85.11 ± 0.22 (84.75 - 85.31)	-0.0055 ± 0.31 (-0.28 - 0.28)	-0.73,0.72	0.986	(82.91 - 86.78) [81.08,88.80]

Table 9. Statistical Summary of Site 4 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Proximate						
Moisture (% FW)	12.40 ± 0.27 (12.10 - 12.80)	12.77 ± 0.27 (12.10 - 13.10)	-0.37 ± 0.38 (-1.00 - 0.20)	-1.23,0.50	0.357	(7.60 - 15.30) [0.45,19.52]
Protein (% DW)	10.31 ± 0.15 (10.26 - 10.35)	10.39 ± 0.15 (10.33 - 10.49)	-0.078 ± 0.21 (-0.19 - 0.019)	-0.57,0.42	0.725	(9.33 - 11.82) [7.54,13.13]
Total Fat (% DW)	3.25 ± 0.12 (3.19 - 3.28)	3.15 ± 0.12 (3.05 - 3.35)	0.097 ± 0.16 (-0.069 - 0.23)	-0.27,0.47	0.562	(2.66 - 3.71) [2.20,4.55]
Vitamin						
Folic Acid (mg/kg DW)	0.27 ± 0.017 (0.26 - 0.28)	0.26 ± 0.017 (0.23 - 0.29)	0.0069 ± 0.020 (-0.012 - 0.045)	-0.040,0.053	0.740	(0.13 - 0.45) [0.012,0.69]
Niacin (mg/kg DW)	31.47 ± 0.86 (30.39 - 33.52)	30.38 ± 0.86 (30.26 - 30.49)	1.09 ± 1.21 (-0.10 - 3.15)	-1.70,3.88	0.394	(16.17 - 29.19) [6.97,37.83]
Vitamin B1 (mg/kg DW)	3.20 ± 0.13 (3.07 - 3.44)	2.98 ± 0.13 (2.76 - 3.22)	0.22 ± 0.18 (-0.15 - 0.68)	-0.21,0.64	0.274	(2.19 - 5.60) [0.37,6.35]
Vitamin B2 (mg/kg DW)	1.25 ± 0.065 (1.24 - 1.26)	1.45 ± 0.065 (1.30 - 1.55)	-0.20 ± 0.092 (-0.30 - -0.049)	-0.41,0.017	0.066	(1.34 - 1.91) [0.91,2.30]
Vitamin B6 (mg/kg DW)	6.36 ± 0.29 (6.15 - 6.47)	6.02 ± 0.29 (5.37 - 6.44)	0.34 ± 0.39 (-0.088 - 1.10)	-0.57,1.25	0.412	(5.08 - 7.47) [3.12,9.30]

Table 9. Statistical Summary of Site 4 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Vitamin						
Vitamin E (mg/kg DW)	6.88 ± 0.55 (6.19 - 7.28)	6.95 ± 0.55 (6.73 - 7.23)	-0.074 ± 0.74 (-1.04 - 0.55)	-1.78,1.63	0.923	(2.71 - 13.94) [0,20.49]
Antinutrient						
Phytic Acid (% DW)	0.60 ± 0.058 (0.53 - 0.73)	0.61 ± 0.058 (0.56 - 0.68)	-0.0070 ± 0.077 (-0.15 - 0.14)	-0.18,0.17	0.929	(0.50 - 0.94) [0.21,1.22]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	2119.34 ± 94.97 (2041.28 - 2200.68)	2090.08 ± 94.97 (2071.35 - 2116.04)	29.26 ± 134.31 (-30.06 - 84.64)	-280.47,338.99	0.833	(1412.68 - 2297.36) [1136.69,2806.24]
p-Coumaric Acid (µg/g DW)	196.33 ± 6.94 (184.63 - 212.09)	177.32 ± 6.94 (172.92 - 180.67)	19.01 ± 9.81 (6.27 - 39.16)	-3.62,41.64	0.088	(99.30 - 285.75) [0,378.57]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 10. Statistical Summary of Site 5 Maize Forage Fiber, Calcium, Phosphorus, and Proximate Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fiber						
Acid Detergent Fiber (% DW)	31.31 ± 1.70 (26.92 - 34.93)	23.58 ± 1.70 (23.06 - 24.48)	7.73 ± 2.40 (3.72 - 10.45)	2.20,13.26	0.012	(26.72 - 38.94) [16.76,43.76]
Neutral Detergent Fiber (% DW)	43.21 ± 2.11 (40.07 - 46.82)	37.87 ± 2.11 (35.06 - 41.38)	5.34 ± 1.98 (5.00 - 5.58)	0.78,9.90	0.027	(33.70 - 46.74) [25.94,55.67]
Mineral						
Calcium (% DW)	0.18 ± 0.014 (0.17 - 0.21)	0.15 ± 0.014 (0.14 - 0.17)	0.030 ± 0.018 (-0.0023 - 0.063)	-0.011,0.071	0.131	(0.11 - 0.29) [0.016,0.38]
Phosphorus (% DW)	0.27 ± 0.019 (0.24 - 0.32)	0.22 ± 0.019 (0.22 - 0.23)	0.049 ± 0.027 (0.0094 - 0.10)	-0.012,0.11	0.102	(0.14 - 0.25) [0.071,0.32]
Proximate						
Ash (% DW)	3.19 ± 0.29 (2.51 - 3.61)	2.97 ± 0.29 (2.59 - 3.38)	0.22 ± 0.39 (-0.87 - 0.86)	-0.67,1.12	0.582	(3.40 - 5.45) [1.93,6.31]
Carbohydrates (% DW)	87.86 ± 0.91 (85.66 - 89.13)	87.82 ± 0.91 (86.94 - 88.77)	0.037 ± 0.85 (-1.28 - 1.03)	-1.93,2.00	0.966	(84.88 - 88.39) [83.05,90.74]
Moisture (% FW)	71.33 ± 1.21 (70.10 - 73.10)	69.93 ± 1.21 (69.20 - 71.00)	1.40 ± 1.40 (-0.90 - 3.50)	-1.84,4.64	0.348	(64.90 - 77.40) [57.62,86.45]
Protein (% DW)	7.50 ± 0.49 (6.65 - 8.49)	7.50 ± 0.49 (7.30 - 7.79)	0.0046 ± 0.67 (-0.65 - 0.70)	-1.53,1.54	0.994	(6.58 - 8.82) [4.78,10.38]
Total Fat (% DW)	1.45 ± 0.42 (0.77 - 2.23)	1.71 ± 0.42 (1.34 - 2.32)	-0.26 ± 0.38 (-0.57 - -0.093)	-1.14,0.61	0.507	(0.58 - 3.11) [0,4.54]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 11. Statistical Summary of Site 5 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Alanine (% DW)	0.86 ± 0.023 (0.82 - 0.89)	0.87 ± 0.023 (0.86 - 0.89)	-0.018 ± 0.027 (-0.069 - 0.023)	-0.081,0.045	0.534	(0.67 - 0.96) [0.48,1.08]
Arginine (% DW)	0.51 ± 0.010 (0.50 - 0.52)	0.50 ± 0.010 (0.49 - 0.51)	0.011 ± 0.014 (0.0046 - 0.016)	-0.021,0.044	0.443	(0.37 - 0.49) [0.33,0.56]
Aspartic acid (% DW)	0.74 ± 0.019 (0.73 - 0.76)	0.74 ± 0.019 (0.72 - 0.76)	0.00048 ± 0.026 (-0.038 - 0.039)	-0.060,0.061	0.985	(0.57 - 0.77) [0.43,0.90]
Cystine (% DW)	0.24 ± 0.0060 (0.23 - 0.25)	0.24 ± 0.0060 (0.24 - 0.25)	-0.0014 ± 0.0059 (-0.014 - 0.0076)	-0.015,0.012	0.817	(0.20 - 0.24) [0.18,0.27]
Glutamic acid (% DW)	2.20 ± 0.063 (2.10 - 2.29)	2.21 ± 0.063 (2.18 - 2.26)	-0.015 ± 0.078 (-0.16 - 0.10)	-0.19,0.16	0.852	(1.71 - 2.41) [1.25,2.75]
Glycine (% DW)	0.41 ± 0.0072 (0.40 - 0.41)	0.40 ± 0.0072 (0.40 - 0.41)	0.0035 ± 0.010 (-0.0074 - 0.015)	-0.020,0.027	0.741	(0.32 - 0.40) [0.28,0.46]
Histidine (% DW)	0.34 ± 0.0072 (0.33 - 0.35)	0.33 ± 0.0072 (0.33 - 0.34)	0.0036 ± 0.0080 (-0.0083 - 0.014)	-0.015,0.022	0.664	(0.26 - 0.33) [0.22,0.38]
Isoleucine (% DW)	0.42 ± 0.011 (0.41 - 0.43)	0.42 ± 0.011 (0.41 - 0.42)	0.0013 ± 0.014 (-0.0052 - 0.0063)	-0.032,0.035	0.930	(0.32 - 0.45) [0.23,0.51]

Table 11. Statistical Summary of Site 5 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Leucine (% DW)	1.50 ± 0.050 (1.41 - 1.57)	1.53 ± 0.050 (1.51 - 1.55)	-0.032 ± 0.055 (-0.13 - 0.044)	-0.16,0.096	0.582	(1.14 - 1.68) [0.77,1.92]
Lysine (% DW)	0.35 ± 0.0073 (0.35 - 0.35)	0.35 ± 0.0073 (0.34 - 0.36)	0.0018 ± 0.010 (-0.012 - 0.011)	-0.022,0.026	0.864	(0.24 - 0.34) [0.20,0.40]
Methionine (% DW)	0.24 ± 0.0066 (0.23 - 0.24)	0.23 ± 0.0066 (0.23 - 0.24)	0.0062 ± 0.0054 (-0.0037 - 0.017)	-0.0063,0.019	0.284	(0.17 - 0.22) [0.14,0.25]
Phenylalanine (% DW)	0.58 ± 0.018 (0.55 - 0.61)	0.59 ± 0.018 (0.58 - 0.60)	-0.0084 ± 0.020 (-0.054 - 0.032)	-0.055,0.039	0.692	(0.45 - 0.65) [0.32,0.73]
Proline (% DW)	0.95 ± 0.026 (0.90 - 1.00)	0.97 ± 0.026 (0.96 - 0.98)	-0.016 ± 0.028 (-0.064 - 0.026)	-0.081,0.050	0.598	(0.83 - 1.11) [0.68,1.21]
Serine (% DW)	0.56 ± 0.016 (0.54 - 0.59)	0.57 ± 0.016 (0.55 - 0.60)	-0.010 ± 0.023 (-0.052 - 0.034)	-0.063,0.043	0.664	(0.45 - 0.62) [0.34,0.71]
Threonine (% DW)	0.33 ± 0.012 (0.30 - 0.35)	0.34 ± 0.012 (0.33 - 0.35)	-0.0095 ± 0.014 (-0.032 - 0.012)	-0.042,0.023	0.524	(0.29 - 0.37) [0.24,0.41]
Tryptophan (% DW)	0.060 ± 0.0015 (0.055 - 0.064)	0.058 ± 0.0015 (0.057 - 0.059)	0.0016 ± 0.0021 (-0.0038 - 0.0072)	-0.0031,0.0064	0.449	(0.043 - 0.059) [0.032,0.072]

Table 11. Statistical Summary of Site 5 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.35 ± 0.051 (0.32 - 0.42)	0.37 ± 0.051 (0.25 - 0.42)	-0.022 ± 0.071 (-0.21 - 0.14)	-0.19,0.14	0.764	(0.25 - 0.40) [0.17,0.52]
Valine (% DW)	0.54 ± 0.013 (0.53 - 0.55)	0.54 ± 0.013 (0.53 - 0.55)	0.0068 ± 0.016 (-0.015 - 0.021)	-0.030,0.044	0.682	(0.42 - 0.55) [0.35,0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	9.00 ± 0.026 (8.98 - 9.03)	8.97 ± 0.026 (8.94 - 9.01)	0.022 ± 0.037 (-0.032 - 0.064)	-0.065,0.11	0.575	(9.10 - 12.55) [6.12,15.67]
16:1 Palmitoleic (% Total FA)	0.13 ± 0.014 (0.13 - 0.14)	0.11 ± 0.014 (0.048 - 0.14)	0.026 ± 0.018 (-0.0012 - 0.079)	-0.015,0.067	0.177	(0.050 - 0.19) [0,0.28]
18:0 Stearic (% Total FA)	1.84 ± 0.012 (1.82 - 1.86)	1.81 ± 0.012 (1.80 - 1.82)	0.031 ± 0.016 (0.017 - 0.059)	-0.0058,0.068	0.087	(1.57 - 2.45) [0.86,2.98]
18:1 Oleic (% Total FA)	24.07 ± 0.29 (23.38 - 24.53)	24.28 ± 0.29 (23.98 - 24.85)	-0.22 ± 0.38 (-1.48 - 0.51)	-1.0,0.67	0.588	(21.17 - 35.33) [7.51,46.46]
18:2 Linoleic (% Total FA)	62.98 ± 0.30 (62.56 - 63.61)	62.86 ± 0.30 (62.37 - 63.16)	0.12 ± 0.37 (-0.60 - 1.24)	-0.74,0.98	0.762	(50.33 - 63.59) [39.41,76.74]
18:3 Linolenic (% Total FA)	1.15 ± 0.012 (1.13 - 1.17)	1.16 ± 0.012 (1.15 - 1.18)	-0.0082 ± 0.015 (-0.049 - 0.016)	-0.042,0.026	0.596	(0.93 - 1.52) [0.63,1.77]

Table 11. Statistical Summary of Site 5 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.38 ± 0.0033 (0.38 - 0.39)	0.37 ± 0.0033 (0.37 - 0.38)	0.012 ± 0.0046 (0.0058 - 0.022)	0.00099,0.022	0.035	(0.32 - 0.47) [0.23,0.54]
20:1 Eicosenoic (% Total FA)	0.28 ± 0.0021 (0.28 - 0.28)	0.28 ± 0.0021 (0.27 - 0.28)	0.0038 ± 0.0029 (0.0022 - 0.0049)	-0.0027,0.010	0.215	(0.23 - 0.32) [0.15,0.39]
22:0 Behenic (% Total FA)	0.17 ± 0.012 (0.15 - 0.20)	0.16 ± 0.012 (0.14 - 0.17)	0.013 ± 0.012 (-0.0020 - 0.029)	-0.015,0.040	0.311	(0.12 - 0.19) [0.081,0.23]
Fiber						
Acid Detergent Fiber (% DW)	5.99 ± 0.52 (5.33 - 7.24)	5.27 ± 0.52 (4.17 - 6.22)	0.72 ± 0.74 (-0.84 - 3.07)	-0.98,2.42	0.358	(4.11 - 6.33) [2.77,7.56]
Neutral Detergent Fiber (% DW)	9.48 ± 0.42 (8.87 - 9.79)	8.87 ± 0.42 (8.57 - 9.44)	0.61 ± 0.59 (0.26 - 1.22)	-0.75,1.96	0.334	(8.20 - 11.30) [5.93,13.63]
Total Dietary Fiber (% DW)	14.34 ± 0.34 (13.80 - 14.94)	13.82 ± 0.34 (13.50 - 14.32)	0.52 ± 0.24 (0.30 - 0.65)	-0.035,1.07	0.062	(12.99 - 18.03) [9.20,20.27]
Mineral						
Calcium (% DW)	0.0049 ± 0.00016 (0.0048 - 0.0049)	0.0046 ± 0.00016 (0.0045 - 0.0047)	0.00026 ± 0.00023 (0.00013 - 0.00037)	-0.00027,0.00079	0.288	(0.0031 - 0.0049) [0.0016,0.0059]
Copper (mg/kg DW)	1.35 ± 0.65 (1.33 - 1.39)	2.68 ± 0.65 (1.84 - 4.29)	-1.32 ± 0.86 (-2.96 - -0.51)	-3.31,0.66	0.163	(1.15 - 3.56) [0,4.20]

Table 11. Statistical Summary of Site 5 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Mineral						
Iron (mg/kg DW)	21.37 ± 0.84 (20.59 - 23.76)	25.74 ± 0.84 (22.83 - 28.26)	-4.38 ± 1.19 (-6.50 - -2.24)	-7.12,-1.63	0.006	(18.04 - 29.22) [8.88,34.51]
Magnesium (% DW)	0.13 ± 0.0024 (0.13 - 0.14)	0.13 ± 0.0024 (0.13 - 0.14)	-0.0014 ± 0.0028 (-0.0046 - 0.00096)	-0.0080,0.0052	0.636	(0.099 - 0.14) [0.075,0.17]
Manganese (mg/kg DW)	6.56 ± 0.17 (6.09 - 6.85)	6.52 ± 0.17 (6.38 - 6.66)	0.039 ± 0.17 (-0.29 - 0.32)	-0.34,0.42	0.817	(5.56 - 8.64) [3.17,9.99]
Phosphorus (% DW)	0.35 ± 0.0055 (0.34 - 0.36)	0.35 ± 0.0055 (0.35 - 0.36)	-0.00071 ± 0.0067 (-0.011 - 0.0058)	-0.016,0.015	0.917	(0.25 - 0.37) [0.18,0.45]
Potassium (% DW)	0.36 ± 0.0041 (0.35 - 0.36)	0.35 ± 0.0041 (0.35 - 0.35)	0.0040 ± 0.0058 (-0.0029 - 0.011)	-0.0092,0.017	0.503	(0.32 - 0.40) [0.26,0.46]
Zinc (mg/kg DW)	22.13 ± 0.55 (21.25 - 22.95)	22.25 ± 0.55 (21.76 - 22.92)	-0.11 ± 0.78 (-0.81 - 1.19)	-1.90,1.68	0.889	(16.72 - 34.04) [7.16,38.55]
Proximate						
Ash (% DW)	1.43 ± 0.048 (1.37 - 1.53)	1.44 ± 0.048 (1.31 - 1.51)	-0.0025 ± 0.055 (-0.099 - 0.063)	-0.13,0.12	0.964	(1.12 - 1.62) [0.74,1.96]
Carbohydrates (% DW)	84.26 ± 0.19 (83.99 - 84.59)	83.80 ± 0.19 (83.58 - 84.03)	0.46 ± 0.14 (0.41 - 0.56)	0.15,0.78	0.009	(82.91 - 86.78) [81.08,88.80]

Table 11. Statistical Summary of Site 5 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Proximate						
Moisture (% FW)	8.89 ± 0.10 (8.71 - 9.01)	8.60 ± 0.10 (8.36 - 8.89)	0.29 ± 0.14 (-0.18 - 0.65)	-0.039,0.61	0.076	(7.60 - 15.30) [0.45,19.52]
Protein (% DW)	11.15 ± 0.20 (10.83 - 11.43)	11.31 ± 0.20 (11.15 - 11.52)	-0.15 ± 0.25 (-0.69 - 0.19)	-0.72,0.42	0.558	(9.33 - 11.82) [7.54,13.13]
Total Fat (% DW)	3.15 ± 0.098 (3.05 - 3.21)	3.46 ± 0.098 (3.14 - 3.68)	-0.31 ± 0.14 (-0.50 - 0.070)	-0.63,0.0082	0.054	(2.66 - 3.71) [2.20,4.55]
Vitamin						
Folic Acid (mg/kg DW)	0.39 ± 0.025 (0.32 - 0.48)	0.35 ± 0.025 (0.32 - 0.37)	0.041 ± 0.035 (-0.043 - 0.11)	-0.040,0.12	0.276	(0.13 - 0.45) [0.012,0.69]
Niacin (mg/kg DW)	26.78 ± 0.70 (25.72 - 28.00)	25.71 ± 0.70 (24.93 - 26.19)	1.07 ± 0.90 (-0.47 - 3.07)	-1.00,3.14	0.268	(16.17 - 29.19) [6.97,37.83]
Vitamin B1 (mg/kg DW)	3.11 ± 0.49 (2.96 - 3.40)	2.99 ± 0.49 (2.85 - 3.06)	0.12 ± 0.69 (-0.088 - 0.34)	-1.47,1.71	0.866	(2.19 - 5.60) [0.37,6.35]
Vitamin B2 (mg/kg DW)	1.52 ± 0.089 (1.44 - 1.61)	1.39 ± 0.089 (1.16 - 1.52)	0.13 ± 0.13 (-0.038 - 0.45)	-0.16,0.42	0.322	(1.34 - 1.91) [0.91,2.30]
Vitamin B6 (mg/kg DW)	5.68 ± 0.30 (5.28 - 6.00)	6.11 ± 0.30 (5.95 - 6.38)	-0.43 ± 0.43 (-0.72 - -0.19)	-1.42,0.56	0.348	(5.08 - 7.47) [3.12,9.30]

Table 11. Statistical Summary of Site 5 Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Vitamin						
Vitamin E (mg/kg DW)	6.83 ± 0.81 (5.55 - 8.62)	8.01 ± 0.81 (6.35 - 9.02)	-1.18 ± 1.15 (-2.35 - -0.40)	-3.82,1.46	0.331	(2.71 - 13.94) [0,20.49]
Antinutrient						
Phytic Acid (% DW)	0.85 ± 0.018 (0.85 - 0.86)	0.86 ± 0.018 (0.83 - 0.88)	-0.010 ± 0.023 (-0.031 - 0.025)	-0.064,0.044	0.676	(0.50 - 0.94) [0.21,1.22]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	2026.73 ± 69.44 (1954.76 - 2092.23)	1932.90 ± 69.44 (1898.73 - 1979.22)	93.82 ± 91.31 (-24.47 - 171.48)	-116.73,304.37	0.334	(1412.68 - 2297.36) [1136.69,2806.24]
p-Coumaric Acid (µg/g DW)	218.38 ± 10.54 (187.79 - 253.04)	185.63 ± 10.54 (182.20 - 189.17)	32.75 ± 12.71 (-1.39 - 70.84)	3.44,62.07	0.032	(99.30 - 285.75) [0,378.57]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 12. Statistical Summary of Combined Site Maize Forage Fiber, Calcium, Phosphorus, and Proximate Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference(Test minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Fiber						
Acid Detergent Fiber (% DW)	28.95 ± 1.69 (22.60 - 35.85)	27.26 ± 1.69 (19.93 - 35.59)	1.69 ± 1.18 (-6.22 - 10.45)	-0.81,4.19	0.170	(26.72 - 38.94) [16.76,43.76]
Neutral Detergent Fiber (% DW)	39.69 ± 1.32 (33.99 - 46.82)	37.60 ± 1.32 (31.44 - 43.96)	2.09 ± 1.40 (-3.47 - 7.47)	-0.88,5.05	0.155	(33.70 - 46.74) [25.94,55.67]
Mineral						
Calcium (% DW)	0.20 ± 0.019 (0.16 - 0.24)	0.19 ± 0.019 (0.13 - 0.28)	0.0066 ± 0.011 (-0.036 - 0.063)	-0.017,0.031	0.569	(0.11 - 0.29) [0.016,0.38]
Phosphorus (% DW)	0.25 ± 0.011 (0.22 - 0.32)	0.21 ± 0.011 (0.15 - 0.25)	0.040 ± 0.014 (-0.0019 - 0.13)	0.011,0.069	0.010	(0.14 - 0.25) [0.071,0.32]
Proximate						
Ash (% DW)	3.70 ± 0.27 (2.51 - 4.67)	3.90 ± 0.27 (2.59 - 5.10)	-0.20 ± 0.21 (-1.72 - 0.97)	-0.65,0.25	0.356	(3.40 - 5.45) [1.93,6.31]
Carbohydrates (% DW)	86.90 ± 0.43 (84.93 - 89.13)	86.69 ± 0.43 (84.36 - 89.57)	0.21 ± 0.53 (-4.23 - 4.41)	-0.91,1.33	0.697	(84.88 - 88.39) [83.05,90.74]
Moisture (% FW)	72.20 ± 1.35 (68.50 - 75.40)	71.53 ± 1.35 (65.90 - 76.80)	0.67 ± 0.52 (-3.50 - 4.20)	-0.44,1.77	0.220	(64.90 - 77.40) [57.62,86.45]
Protein (% DW)	7.82 ± 0.27 (6.34 - 8.98)	7.70 ± 0.27 (6.06 - 8.87)	0.13 ± 0.26 (-2.32 - 2.35)	-0.43,0.68	0.635	(6.58 - 8.82) [4.78,10.38]
Total Fat (% DW)	1.57 ± 0.24 (0.63 - 3.17)	1.71 ± 0.24 (0.77 - 2.91)	-0.13 ± 0.23 (-2.28 - 1.95)	-0.59,0.32	0.558	(0.58 - 3.11) [0,4.54]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 13. Statistical Summary of Combined Site Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Alanine (% DW)	0.77 ± 0.019 (0.64 - 0.89)	0.78 ± 0.039 (0.67 - 0.89)	-0.0070 ± 0.019 (-0.13 - 0.089)	-0.046,0.032	0.709	(0.67 - 0.96) [0.48,1.08]
Arginine (% DW)	0.48 ± 0.013 (0.38 - 0.52)	0.47 ± 0.013 (0.41 - 0.51)	0.011 ± 0.012 (-0.090 - 0.062)	-0.014,0.036	0.361	(0.37 - 0.49) [0.33,0.56]
Aspartic acid (% DW)	0.68 ± 0.029 (0.56 - 0.78)	0.67 ± 0.029 (0.60 - 0.76)	0.0038 ± 0.015 (-0.11 - 0.078)	-0.028,0.036	0.804	(0.57 - 0.77) [0.43,0.90]
Cystine (% DW)	0.23 ± 0.0057 (0.20 - 0.26)	0.23 ± 0.0057 (0.21 - 0.25)	0.0023 ± 0.0038 (-0.022 - 0.023)	-0.0057,0.010	0.554	(0.20 - 0.24) [0.18,0.27]
Glutamic acid (% DW)	1.97 ± 0.097 (1.63 - 2.29)	1.99 ± 0.097 (1.70 - 2.26)	-0.012 ± 0.049 (-0.33 - 0.24)	-0.11,0.091	0.809	(1.71 - 2.41) [1.25,2.75]
Glycine (% DW)	0.38 ± 0.0087 (0.32 - 0.41)	0.38 ± 0.0087 (0.36 - 0.41)	0.0042 ± 0.0071 (-0.067 - 0.035)	-0.011,0.019	0.566	(0.32 - 0.40) [0.28,0.46]
Histidine (% DW)	0.31 ± 0.011 (0.25 - 0.35)	0.31 ± 0.011 (0.28 - 0.34)	0.0027 ± 0.0055 (-0.050 - 0.030)	-0.0090,0.014	0.632	(0.26 - 0.33) [0.22,0.38]
Isoleucine (% DW)	0.36 ± 0.018 (0.30 - 0.43)	0.36 ± 0.018 (0.30 - 0.42)	-0.00003 ± 0.0088 (-0.056 - 0.041)	-0.019,0.019	0.997	(0.32 - 0.45) [0.23,0.51]

Table 13. Statistical Summary of Combined Site Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Leucine (% DW)	1.31 ± 0.077 (1.09 - 1.57)	1.32 ± 0.077 (1.08 - 1.55)	-0.014 ± 0.036 (-0.21 - 0.16)	-0.089,0.062	0.700	(1.14 - 1.68) [0.77,1.92]
Lysine (% DW)	0.33 ± 0.0097 (0.26 - 0.36)	0.32 ± 0.0097 (0.29 - 0.36)	0.0088 ± 0.0078 (-0.056 - 0.033)	-0.0077,0.025	0.273	(0.24 - 0.34) [0.20,0.40]
Methionine (% DW)	0.23 ± 0.0064 (0.20 - 0.27)	0.22 ± 0.0064 (0.20 - 0.24)	0.0038 ± 0.0047 (-0.017 - 0.028)	-0.0061,0.014	0.427	(0.17 - 0.22) [0.14,0.25]
Phenylalanine (% DW)	0.51 ± 0.028 (0.43 - 0.61)	0.52 ± 0.028 (0.43 - 0.60)	-0.0012 ± 0.013 (-0.080 - 0.067)	-0.029,0.026	0.925	(0.45 - 0.65) [0.32,0.73]
Proline (% DW)	0.93 ± 0.030 (0.79 - 1.05)	0.93 ± 0.030 (0.83 - 1.01)	0.0034 ± 0.019 (-0.15 - 0.10)	-0.037,0.044	0.861	(0.83 - 1.11) [0.68,1.21]
Serine (% DW)	0.52 ± 0.022 (0.44 - 0.61)	0.52 ± 0.022 (0.46 - 0.60)	-0.0046 ± 0.012 (-0.087 - 0.058)	-0.030,0.021	0.703	(0.45 - 0.62) [0.34,0.71]
Threonine (% DW)	0.33 ± 0.010 (0.27 - 0.37)	0.33 ± 0.010 (0.29 - 0.36)	0.00063 ± 0.0074 (-0.052 - 0.039)	-0.015,0.016	0.933	(0.29 - 0.37) [0.24,0.41]
Tryptophan (% DW)	0.056 ± 0.0018 (0.048 - 0.064)	0.056 ± 0.0018 (0.045 - 0.063)	0.00031 ± 0.0013 (-0.0055 - 0.0072)	-0.0025,0.0031	0.817	(0.043 - 0.059) [0.032,0.072]

Table 13. Statistical Summary of Combined Site Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.37 ± 0.015 (0.22 - 0.43)	0.36 ± 0.015 (0.24 - 0.42)	0.0088 ± 0.016 (-0.21 - 0.14)	-0.026,0.043	0.596	(0.25 - 0.40) [0.17,0.52]
Valine (% DW)	0.49 ± 0.020 (0.40 - 0.55)	0.49 ± 0.020 (0.43 - 0.55)	0.0034 ± 0.010 (-0.084 - 0.055)	-0.019,0.026	0.748	(0.42 - 0.55) [0.35,0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	9.19 ± 0.060 (8.98 - 9.46)	9.12 ± 0.060 (8.91 - 9.34)	0.071 ± 0.049 (-0.14 - 0.33)	-0.034,0.18	0.171	(9.10 - 12.55) [6.12,15.67]
16:1 Palmitoleic (% Total FA)	0.13 ± 0.0058 (0.11 - 0.14)	0.12 ± 0.0058 (0.048 - 0.14)	0.0022 ± 0.0054 (-0.012 - 0.079)	-0.0093,0.014	0.696	(0.050 - 0.19) [0,0.28]
18:0 Stearic (% Total FA)	1.89 ± 0.021 (1.79 - 2.03)	1.82 ± 0.021 (1.76 - 1.87)	0.072 ± 0.021 (-0.055 - 0.18)	0.028,0.12	0.002	(1.57 - 2.45) [0.86,2.98]
18:1 Oleic (% Total FA)	24.96 ± 0.34 (23.38 - 25.75)	24.84 ± 0.34 (23.62 - 26.66)	0.12 ± 0.20 (-1.48 - 1.15)	-0.32,0.55	0.574	(21.17 - 35.33) [7.51,46.46]
18:2 Linoleic (% Total FA)	61.82 ± 0.40 (60.85 - 63.61)	62.07 ± 0.40 (60.51 - 63.41)	-0.25 ± 0.23 (-1.62 - 1.24)	-0.73,0.24	0.292	(50.33 - 63.59) [39.41,76.74]
18:3 Linolenic (% Total FA)	1.19 ± 0.027 (1.12 - 1.23)	1.22 ± 0.027 (1.15 - 1.43)	-0.028 ± 0.016 (-0.23 - 0.036)	-0.063,0.0061	0.099	(0.93 - 1.52) [0.63,1.77]

Table 13. Statistical Summary of Combined Site Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.39 ± 0.0062 (0.36 - 0.42)	0.38 ± 0.0062 (0.36 - 0.40)	0.013 ± 0.0031 (-0.019 - 0.032)	0.0063,0.019	<0.001	(0.32 - 0.47) [0.23,0.54]
20:1 Eicosenoic (% Total FA)	0.28 ± 0.0040 (0.26 - 0.29)	0.28 ± 0.0040 (0.25 - 0.29)	0 ± 0.0024 (-0.014 - 0.011)	-0.0051,0.0051	0.999	(0.23 - 0.32) [0.15,0.39]
22:0 Behenic (% Total FA)	0.16 ± 0.0050 (0.13 - 0.20)	0.15 ± 0.0050 (0.13 - 0.18)	0.0027 ± 0.0062 (-0.019 - 0.029)	-0.010,0.016	0.665	(0.12 - 0.19) [0.081,0.23]
Fiber						
Acid Detergent Fiber (% DW)	5.48 ± 0.19 (3.82 - 7.24)	5.27 ± 0.19 (4.17 - 7.00)	0.21 ± 0.25 (-3.18 - 3.07)	-0.30,0.72	0.410	(4.11 - 6.33) [2.77,7.56]
Neutral Detergent Fiber (% DW)	10.06 ± 0.37 (8.59 - 12.08)	9.75 ± 0.37 (8.48 - 11.75)	0.31 ± 0.34 (-2.26 - 2.05)	-0.41,1.03	0.370	(8.20 - 11.30) [5.93,13.63]
Total Dietary Fiber (% DW)	15.17 ± 0.47 (13.39 - 17.02)	14.67 ± 0.47 (12.82 - 17.62)	0.50 ± 0.54 (-3.61 - 4.20)	-0.66,1.65	0.375	(12.99 - 18.03) [9.20,20.27]
Mineral						
Calcium (% DW)	0.0050 ± 0.00034 (0.0038 - 0.0066)	0.0049 ± 0.00034 (0.0040 - 0.0059)	0.00016 ± 0.00011 (-0.00027 - 0.00090)	-0.00008,0.00040	0.180	(0.0031 - 0.0049) [0.0016,0.0059]
Copper (mg/kg DW)	1.74 ± 0.38 (1.33 - 2.38)	2.07 ± 0.37 (1.26 - 4.54)	-0.33 ± 0.53 (-2.96 - 0.78)	-1.45,0.79	0.547	(1.15 - 3.56) [0,4.20]

Table 13. Statistical Summary of Combined Site Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference(Test minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Mineral						
Iron (mg/kg DW)	21.40 ± 1.00 (19.23 - 25.23)	22.20 ± 0.99 (19.03 - 28.26)	-0.80 ± 0.67 (-6.50 - 5.90)	-2.22,0.62	0.250	(18.04 - 29.22) [8.88,34.51]
Magnesium (% DW)	0.12 ± 0.0043 (0.10 - 0.14)	0.12 ± 0.0043 (0.11 - 0.14)	-0.00028 ± 0.0021 (-0.018 - 0.011)	-0.0047,0.0041	0.893	(0.099 - 0.14) [0.075,0.17]
Manganese (mg/kg DW)	6.79 ± 0.29 (5.43 - 9.32)	6.51 ± 0.29 (5.57 - 8.00)	0.28 ± 0.21 (-1.54 - 2.36)	-0.18,0.73	0.213	(5.56 - 8.64) [3.17,9.99]
Phosphorus (% DW)	0.33 ± 0.0095 (0.27 - 0.36)	0.33 ± 0.0095 (0.29 - 0.36)	-0.00039 ± 0.0043 (-0.038 - 0.026)	-0.0087,0.0095	0.929	(0.25 - 0.37) [0.18,0.45]
Potassium (% DW)	0.36 ± 0.0065 (0.32 - 0.40)	0.36 ± 0.0065 (0.34 - 0.40)	-0.0032 ± 0.0042 (-0.030 - 0.035)	-0.0052,0.012	0.450	(0.32 - 0.40) [0.26,0.46]
Zinc (mg/kg DW)	22.05 ± 1.14 (18.91 - 26.89)	21.91 ± 1.14 (18.81 - 26.04)	0.14 ± 0.51 (-3.37 - 3.19)	-0.94,1.22	0.788	(16.72 - 34.04) [7.16,38.55]
Proximate						
Ash (% DW)	1.41 ± 0.036 (1.25 - 1.56)	1.39 ± 0.036 (1.28 - 1.51)	0.014 ± 0.041 (-0.11 - 0.13)	-0.072,0.10	0.734	(1.12 - 1.62) [0.74,1.96]
Carbohydrates (% DW)	84.85 ± 0.42 (83.29 - 86.52)	84.96 ± 0.42 (83.58 - 86.22)	-0.11 ± 0.18 (-1.42 - 0.84)	-0.50,0.28	0.562	(82.91 - 86.78) [81.08,88.80]

Table 13. Statistical Summary of Combined Site Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

			Difference(Test minus Control)			
Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Proximate						
Moisture (% FW)	9.52 ± 0.77 (7.89 - 12.80)	9.50 ± 0.77 (7.86 - 13.10)	0.021 ± 0.22 (-1.00 - 0.87)	-0.44,0.48	0.923	(7.60 - 15.30) [0.45,19.52]
Protein (% DW)	10.43 ± 0.42 (8.54 - 11.98)	10.36 ± 0.42 (9.22 - 11.52)	0.070 ± 0.19 (-1.26 - 1.28)	-0.34,0.48	0.725	(9.33 - 11.82) [7.54,13.13]
Total Fat (% DW)	3.32 ± 0.069 (3.05 - 3.89)	3.29 ± 0.069 (3.05 - 3.75)	0.025 ± 0.089 (-0.50 - 0.29)	-0.16,0.21	0.784	(2.66 - 3.71) [2.20,4.55]
Vitamin						
Folic Acid (mg/kg DW)	0.35 ± 0.037 (0.26 - 0.48)	0.36 ± 0.037 (0.23 - 0.53)	-0.0080 ± 0.022 (-0.11 - 0.11)	-0.054,0.038	0.717	(0.13 - 0.45) [0.012,0.69]
Niacin (mg/kg DW)	30.08 ± 1.11 (25.72 - 34.84)	29.59 ± 1.11 (24.93 - 35.75)	0.48 ± 0.65 (-4.44 - 5.64)	-0.82,1.79	0.461	(16.17 - 29.19) [6.97,37.83]
Vitamin B1 (mg/kg DW)	3.07 ± 0.13 (2.39 - 3.44)	2.94 ± 0.13 (2.39 - 3.36)	0.13 ± 0.17 (-0.66 - 0.68)	-0.24,0.49	0.474	(2.19 - 5.60) [0.37,6.35]
Vitamin B2 (mg/kg DW)	1.42 ± 0.046 (1.24 - 1.65)	1.42 ± 0.046 (1.16 - 1.61)	0.0015 ± 0.050 (-0.30 - 0.45)	-0.099,0.10	0.976	(1.34 - 1.91) [0.91,2.30]
Vitamin B6 (mg/kg DW)	6.22 ± 0.23 (5.28 - 6.99)	6.26 ± 0.23 (5.37 - 6.80)	-0.036 ± 0.18 (-0.72 - 1.10)	-0.41,0.34	0.838	(5.08 - 7.47) [3.12,9.30]

Table 13. Statistical Summary of Combined Site Maize Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for Test MON 89034 vs. the Conventional Control LH198 x LH172

Analytical Component	Test Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference(Test minus Control) Mean ± S.E. (Range)	95% CI (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[1]]
Vitamin						
Vitamin E (mg/kg DW)	6.77 ± 0.42 (5.55 - 8.62)	6.63 ± 0.42 (2.72 - 9.02)	0.14 ± 0.36 (-2.35 - 3.83)	-0.64,0.91	0.714	(2.71 - 13.94) [0,20.49]
Antinutrient						
Phytic Acid (% DW)	0.75 ± 0.050 (0.53 - 0.87)	0.73 ± 0.050 (0.56 - 0.88)	0.016 ± 0.027 (-0.15 - 0.18)	-0.037,0.069	0.537	(0.50 - 0.94) [0.21,1.22]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	2131.38 ± 108.09 (1790.25 - 2525.31)	2148.05 ± 108.09 (1878.66 - 2669.85)	-16.67 ± 50.08 (-330.17 - 264.79)	-116.98,83.65	0.740	(1412.68 - 2297.36) [1136.69,2806.24]
p-Coumaric Acid (µg/g DW)	194.25 ± 7.12 (166.11 - 253.04)	183.96 ± 7.12 (167.76 - 210.13)	10.28 ± 7.08 (-24.37 - 70.84)	-4.73,25.30	0.165	(99.30 - 285.75) [0,378.57]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 14. Literature and ILSI Database Ranges for Components in Corn Forage and Grain

Tissue/ Component[1]	Literature Range[2]	ILSI Range[3]
Forage		
Proximates (% dw)		
Ash	2.43-9.64[a]; 2-6.6[b]	1.527 – 9.638
Carbohydrates	83.2-91.6[b]; 76.5-87.3[a]	76.4 – 92.1
Fat, total	0.35-3.62[b]; 1.42-4.57[a]	0.296 – 4.570
Moisture (% fw)	56.5-80.4[a];55.3-75.3[b]	49.1 – 81.3
Protein	4.98-11.56[a]	3.14 – 11.57
Fiber (% dw)		
Acid detergent fiber (ADF)	18.3-41.0[b]; 17.5-38.3[a]	16.13 – 47.39
Neutral detergent fiber (NDF)	26.4-54.5[b]; 27.9-54.8[a]	20.29 – 63.71
Minerals (% dw)		
Calcium	0.0969-0.3184[b]	0.0714 – 0.5768
Phosphorous	0.1367-0.2914[b]	0.0936 – 0.3704
Grain		
Proximates (% dw)		
Ash	1.1-3.9[d]; 0.89-6.28[b]	0.616 – 6.282
Carbohydrates	77.4-87.2[b]; 82.2-88.1[a]	77.4 – 89.5
Fat, total	3.1-5.7[d]; 2.48-4.81[b]	1.742 – 5.823
Moisture (% fw)	7-23[d]; 8.18-26.2[b]	6.1 – 40.5
Protein	6-12[d]; 9.7-16.1[c]	6.15 – 17.26
Fiber (% dw)		
Acid detergent fiber (ADF)	3.3-4.3[d]; 2.46-11.34[a,b]	1.82 – 11.34
Neutral detergent fiber (NDF)	8.3-11.9[d]; 7.58-	5.59 – 22.64
Total dietary fiber (TDF)	10.99-91[b]41[h]	8.85 – 35.31
Minerals		
Calcium (% dw)	0.01-0.1[d]	0.00127 – 0.02084
Copper (mg/kg dw)	0.9-10[d]	0.73 – 18.50
Iron (mg/kg dw)	1-100[d]	10.42 – 49.07
Magnesium (% dw)	0.09-1[d]	0.0594 – 0.194
Manganese (mg/kg dw)	0.7-54[d]	1.69 – 14.30
Phosphorous (% dw)	0.26-0.75[d]	0.147 – 0.533
Potassium (% dw)	0.32-0.72[d]	0.181 – 0.603
Zinc (mg/kg dw)	12-30[d]	6.5 – 37.2

Table 14. Literature and ILSI Database Ranges for Components in Corn Forage and Grain

Tissue/ Component[1]	Literature Range[2]	ILSI Range[3]
Grain		
Amino Acids	(% dw)	(% dw)
Alanine	n/a	0.439 – 1.393
Arginine	n/a	0.119 – 0.639
Aspartic acid	n/a	0.335 – 1.208
Cystine	n/a	0.123 – 0.514
Glutamic acid	n/a	0.965 – 3.536
Glycine	n/a	0.184 – 0.539
Histidine	n/a	0.137 – 0.434
Isoleucine	n/a	0.179 – 0.692
Leucine	n/a	0.642 – 2.492
Lysine	n/a	0.172 – 0.668
Methionine	n/a	0.124 – 0.468
Phenylalanine	n/a	0.244 – 0.930
Proline	n/a	0.462 – 1.632
Serine	n/a	0.235 – 0.769
Threonine	n/a	0.224 – 0.666
Tryptophan	n/a	0.0271 – 0.215
Tyrosine	n/a	0.103 – 0.642
Valine	n/a	0.266 – 0.855
Fatty Acids	(% total fat)	(% total fatty acid)
16:0 Palmitic	7-19[e]	7.94 – 20.71
16:1 Palmitoleic	1[e]	0.095 – 0.447
18:0 Stearic	1-3[e]	1.02 – 3.40
18:1 Oleic	20-46[e]	17.4 – 40.2
18:2 Linoleic	35-70[e]	36.2 – 66.5
18:3 Linolenic	0.8-2[e]	0.57 – 2.25
20:0 Arachidic	0.1-2[e]	0.279 – 0.965
20:1 Eicosenoic	n/a	0.170 – 1.917
22:0 Behenic	n/a	0.110 – 0.349
Vitamins	(mg/kg dw)	(mg/kg dw)
Folic acid	0.3[d]	0.147 – 1.464
Niacin	9.3-70[d]	10.37 – 46.94
Vitamin B_1	3-8.6[e]	1.26 – 40.00
Vitamin B_2	0.25-5.6[e]	0.50 – 2.36
Vitamin B_6	5.3[d]; 9.6[e]	3.68 – 11.32
Vitamin E	3-12.1[e]; 17-47[d]	1.5 – 68.7

Table 14. Literature and ILSI Database Ranges for Components in Corn Forage and Grain

Tissue/ Component[1]	Literature Range[2]	ILSI Range[3]
Grain		
Anti-Nutrients (% dw)		
Phytic acid	0.48-1.12[a]	0.111 – 1.570
Raffinose	0.08-0.30[e]	0.020 – 0.320
Secondary Metabolites	(µg/g dw)	(µg/g dw)
Ferulic acid	113-1194[f]; 3000[g]	291.9 – 3885.8
p-Coumaric acid	22-75[f]	53.4 – 576.2

[1]fw=fresh weight; dw=dry weight; Niacin =Vitamin B_3; Vitamin B_1 =Thiamine; Vitamin B_2 =Riboflavin; Vitamin B_6 =Pyridoxine

[2]Literature range references: [a]Ridley *et al.*, 2002a. [b]Sidhu *et al.*, 2000a. [c]Jugenheimer, 1976. [d]Watson, 1987. [e]Watson, 1982. [f]Classen *et al.*, 1990. [g]Dowd and Vega, 1996. [h]Choi *et al.*, 1999.

[3]ILSI range is from ILSI Crop Composition Database, 2006.

n/a = not available

Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw