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RECEIVED
12 MAR 2002

Lynda Graf
Product Evaluator
ANZFA
PO Box 7186
Canberra MC ACT 2610

Dear Lynda

AUSTRALIA NEW ZEALAND FOOD AUTHORITY ACT 1991
Ref: A 416 CP4EPSPS gene in Roundup Ready® Corn Line NK603

The attached report (MSL-17672) was recently issued as an amendment from an earlier report (MSL-16278 Volume 5 of the original application) completed in 1999. It is a composition and expression report for NK603 used in most of our submissions. The report required revisions to the statistical analysis after an error was discovered in the combined site statistical program. The change in the statistical analysis resulted in fewer observed differences. As a consequence, the report itself is only slightly modified, with a listing of the actual changes to the original report in the table on page 6. In addition, the text changes are highlighted in blue to be more easily recognized as changes. Please find enclosed the following study:

- Sidhu, R.S. and B.E. Ledesma. 2002. Amended report for MSL16278: Introduced Protein Levels and Compositional Analyses of Roundup Ready® Corn Line NK603 Tissues Produced in 1998 U.S. Field Trials. Monsanto Technical Report **MSL17672** St Louis Mo.

No confidential information is contained in any of the three volumes.

yours sincerely
MONSANTO AUSTRALIA LIMITED

Megan Shaw
Regulatory Product Manager
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07 March 2002

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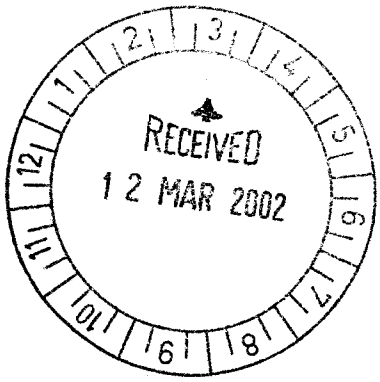

APPLICANT: **Monsanto Australia Limited**

A416
CP4 ESPS gene in Roundup Ready® Corn Line NK603

SUBMISSION: Application to Australia New Zealand Food Authority for the inclusion of corn containing the CP4 EPSPS gene by Monsanto in Standard A18 - Food Derived From Gene Technology

VOLUME: 1 of 1

SUPPORTING INFORMATION

DATE: 07 March 2002

PREPARED BY: Megan Shaw
Regulatory Product Manager

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Study Title

Amended Report for MSL-16278: Introduced Protein Levels and Compositional Analyses of Roundup Ready® Corn Line NK603 Tissues Produced in 1998 U.S. Field Trials

Authors

Ravinder S. Sidhu
Bibiana E. Ledesma

Study Completed On

Amendment 1
February 28, 2002

Performing Laboratories

Monsanto Company
Biotechnology Regulatory Sciences
700 Chesterfield Parkway North
St. Louis, MO 63198

Covance Laboratories, Inc.
Wisconsin Facility
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-17672
Study 99-01-46-38
Covance Study No. 6103-229

Statement of No Data Confidentiality Claims

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)(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."

Company: Monsanto Company

Company Agent: _____

Title: _____

Signature: _____ Date: _____

Statement of Compliance

This study meets GLP requirements of 40 CFR Part 160 (EPA) except for the following:

The reference standards used for compositional analysis were not characterized according to GLP standards and the empty amino acid stock standard bottles were discarded prior to study finalization. This exception had no effect on the integrity or quality of the study because the reference standards were obtained from reputable suppliers.

Submitter: _____ Date: _____

Sponsor: _____ Date: Feb 28, 2002

Study Director: Ravinder S Sidhu Date: Feb 28, 2002

Quality Assurance Statement

Study Title: Amended Report for MSL-16278: Introduced Protein Levels and Compositional Analyses of Roundup Ready® Corn Line NK603 Tissues Produced in 1998 U.S. Field Trials

Study Number: 99-01-46-38

Reviews conducted by the QAU confirm that the final report reflects the raw data.

Following is a list of reviews conducted by the Monsanto AG Regulatory QAU on the study reported herein. Additional reviews conducted by the Quality Assurance Unit of Covance Laboratories are presented in the contract facility report.

Dates Of Inspection / Audit	Phase	Date Reported To: Study Director	Management
08/03/1999	ELISA	08/17/1999	08/17/1999
09/27/1999	Data/Report Audit	09/30/1999	09/30/1999
09/28/1999	Data/Report Audit	09/28/1999	09/28/1999
09/29/1999	Extraction and ELISA	09/30/1999	09/30/1999
09/30/1999	Final Report and Data Audit	10/01/1999	10/01/1999
02/22/2002	Amended Report Audit	02/28/2002	02/28/2002

Kristina Berman
Quality Assurance
Monsanto Regulatory, Monsanto Company

2/28/02
Date

Signatures of Approval

Study Number: 99-01-46-38

Title: Amended Report for MSL-16278: Introduced Protein Levels and Compositional Analyses of Roundup Ready® Corn Line NK603 Tissues Produced in 1998 U.S. Field Trials

Facilities:

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Study Director: Ravinder S. Sidhu

Sponsor Representative: Patrick T. Weston

Contributors: Monsanto (St. Louis): Bibiana E. Ledesma, Susan Riordan, Margaret A. Nemeth and Ravinder S. Sidhu
Covance Laboratories, Inc: Tammy Olson
Certus International, Inc: Roy Sorbet (statistical analysis)

Study Initiation Date: May 26, 1999

Original Study Completion Date: October 1, 1999

Amended Report Completion Date: February 28, 2002

Records Retention: All study specific raw data, protocols, final reports and facility records will be retained at Monsanto, St. Louis, except for raw data and facility records maintained at Covance Laboratories, Inc., Wisconsin Facility.

Sample Storage: Any unused study samples that are not destroyed will be stored at Monsanto, St. Louis.

Signatures of Approval (continued)

Amendments to MSL-16278 Report:

This amendment modifies the final report to reflect revised statistical information for the combined site analysis. The following changes do not affect the quality or integrity of the data.

Item	MSL-17672 Amendment 1 Report	MSL-16278 Original Report	Amendment
1.	Title Page	Title Page	Amended study title, study completion date; added new MSL no., address of statistical facility, Amendment 1
2.	Page 2	Page 2	Revised Statement of No Data Confidentiality Claim
3.	Page 4	Page 4	Added new line to QA Statement: "Amended Report Audit" to list of phases and amended study title
4.	Page 5	Page 5	Added amended study title, statistical facility address, sponsor, "Original Study Completion Date" and "Amended Report Completion Date"
5.	Page 6	Not included	List of Changes in Amended Report
6.	Pages 7 and 8	Pages 6 and 7	Table of Contents - changed pagination
7.	Page 11	Page 10	Reworded text to reflect new statistical information and expression of differences as % of control mean
8.	Page 20	Page 19	Clarified that statistical analyses for NK541, NK543 and NK600 were not reported because lines were dropped for commercial reasons
9.	Page 21	Page 20	Added 'Protocol Amendment #5'
10.	Page 22, 23	Page 21, 22	Reworded text to reflect new statistical information and expression of differences as % of control mean
11.	Table 2, Page 29	Table 2, Page 28	'All Trials' revised to reflect new statistical analysis for combined trials, and differences between test and control expressed as a % of control mean value; corrected typographical error and significant figures
12.	Tables 7 and 8, Pages 44-50	Tables 7 and 8, Pages 43-49	Revised data for 'All Trials' tables to reflect new statistical analysis for combined trials

Signatures of Approval:

Ravinder S Sidhu — Feb 28, 2002
Study Director — Date

Feb 28, 2002
Director, Product Safety Center — Date

Feb 28, 2002
Sponsor — Date

Table of Contents

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Abbreviations

ADF	Acid detergent fiber
AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists Society
CaMV	cauliflower mosaic virus
CTP2	chloroplast transit peptide
DTT	Dithiothreitol
dw	dry weight
e35S	enhanced 35S plant promoter
CP4 EPSPS	a 5-enolpyruvylshikimate-3-phosphate synthase protein isolated from *Agrobacterium* sp. strain CP4 (CP4 EPSPS)
E. coli	*Escherichia coli*
ELISA	Enzyme-linked immunosorbent assay
fwt	fresh weight of tissue
fw	fresh weight
HRP	horseradish peroxidase
LOD	Limit of Detection
LOQ	Limit of Quantitation
NDF or NDFE	Neutral detergent fiber
NOS	Nopaline synthase
OD	Optical density (sample absorbance)
PBST	Phosphate-buffered saline with Tween 20
PCR	Polymerase chain reaction
RR	Roundup Ready®
SOP	Standard Operating Procedure
TBA	Tris-borate with L-ascorbic acid
T/C/R	Test/Control/Reference
TMB	(3,3',5,5' Tetramethylbenzidene) peroxidase substrate
Tris-Cl	Tris(hydroxymethyl)aminomethane hydrochloride
Zmhsp70	*Zea mays* heat-shock protein 70 intron

[Standard abbreviations, e.g., units of measure, according to format described in 'Instructions to Authors' in the Journal of Biological Chemistry]

I. Summary

Monsanto Company has developed Roundup Ready® corn line NK603 which is tolerant to glyphosate (the active ingredient in Roundup herbicide) at the whole plant level. Corn line NK603 contains a 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). Corn plants that demonstrate commercial level tolerance to Roundup® herbicide are called Roundup Ready (RR). The CP4 EPSPS gene from *Agrobacterium* sp. strain CP4 has been completely sequenced and encodes a 47.6-kDa protein consisting of a single polypeptide of 455 amino acids (Padgette *et al.*, 1996). The CP4 EPSPS protein is functionally similar to plant EPSPS enzymes but has a much reduced affinity for glyphosate (Padgette *et al.*, 1993). In nontransgenic plants, glyphosate binds to the plant EPSPS enzyme and blocks the biosynthesis of aromatic amino acids thereby depriving plants of these essential nutrients (Steinrucken and Amrhein, 1980; Haslam, 1993). In Roundup Ready plants, nutritional requirements for normal growth and development are met by the continued action of the glyphosate-tolerant CP4 EPSPS enzyme in the presence of glyphosate. A comprehensive safety assessment of the CP4 EPSPS protein has been described in the literature (Harrison *et al.* 1996).

Four RR corn test lines (NK541, NK543, NK600 and NK603) containing the CP4 EPSPS gene were originally considered for evaluation. However, only data from corn line NK603 is reported as the other test lines were dropped from this study for commercial reasons. Corn line NK603 was produced by transformation of corn tissue with a 6.7-kb linear DNA fragment PV-ZMGT32L derived from the plasmid vector PV-ZMGT32, using a particle acceleration method. Molecular analysis (Deng *et al.*, 1999) has shown that corn line NK603 contains a single DNA insert consisting of two expression cassettes: the first CP4 EPSPS gene cassette, containing the CP4 EPSPS coding sequence under regulation of the rice actin promoter and intron (P-ract1/ract intron), a chloroplast transit peptide (CTP2) sequence, and a nopaline synthase (NOS) 3' polyadenylation sequence; and a second CP4 EPSPS gene cassette, containing the CP4 EPSPS coding sequence under the regulation of the cauliflower mosaic virus (CaMV) enhanced 35S plant promoter (e35S), a maize heat-shock protein 70 (*Zm*hsp70) intron, CTP2 and the NOS 3' polyadenylation sequence.

The purpose of this study was to estimate levels of the CP4 EPSPS protein and to conduct compositional analyses on key corn tissues produced in 1998 U.S. field trials at two replicated sites in Illinois and Ohio (IL2, OH) and at six non-replicated sites in Iowa, Illinois, Indiana and Kansas (IA1, IA2, IA3, IL1, IN and KS). Forage and grain samples collected from corn line NK603 and the nontransgenic parental control line (LH82 x B73) were analyzed by an enzyme-linked immunosorbent assay (ELISA) to estimate the levels of CP4 EPSPS protein present in these tissues. Compositional analyses were conducted to measure proximate (protein, fat, ash, carbohydrate, moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acid, fatty acid, vitamin E, mineral (calcium, copper, iron, magnesium,

manganese, phosphorus, potassium, sodium and zinc), phytic acid and trypsin inhibitor content of grain; and proximate, ADF and NDF content of forage. Statistical analyses were conducted using SAS® software to determine statistically significant differences ($p < 0.05$).

ELISA results showed that mean CP4 EPSPS protein levels in corn line NK603 were comparable for non-replicated sites and replicated sites in forage as well as in grain. Therefore, it is concluded that the CP4 EPSPS protein introduced into corn line NK603 is expressed at approximately the same levels either within site or across geographically dispersed sites.

Fifty-one different compositional components were evaluated for corn line NK603 as part of the safety and nutritional assessment of this product. The values for all the biochemical components assessed were either within the range observed for nontransgenic commercial corn lines, published literature ranges (Jugenheimer, 1976; Watson, 1982; Watson, 1987) or previously reported ranges for nontransgenic corn varieties (Sanders and Patzer, 1995; Sanders *et al.*, 1996a,b; 1997a,b). Data were developed and statistical analyses conducted for three sets of comparisons: analyses for each of the two replicated trials and for a combination of trials at different field sites. Therefore, a total of 153 comparisons were made, 51 comparisons for each of these three statistical analyses. Statistical evaluation showed that there were no statistically significant differences in 135 of the 153 comparisons made between corn line NK603 and the control line. Only one of the statistically significant differences was consistently observed for each of the two replicated trial comparisons and the comparisons across sites. Differences which were observed for only one or two of these comparisons, and not consistently across all three comparisons, are not considered biologically meaningful or relevant. Furthermore, all of these differences were well within reported ranges for corn. The only component for which statistically significant differences were observed across all three statistical evaluations was 18:0 stearic acid in grain. The absolute magnitude of the differences as a percent of the corresponding control mean value for stearic acid ranged between 3.90-5.41%. These values are well within the range of natural variability and the published ranges for stearic acid in corn grain, and therefore, these differences are not considered biologically relevant. Grain and forage from corn line NK603 are considered compositionally equivalent to that of conventional corn.

This data, together with the safe history of use of corn as a common source of animal feed and human food, lead to the conclusion that Roundup Ready corn line NK603 is compositionally equivalent and as safe and nutritious as corn varieties grown commercially today.

II. Introduction

A. Background

Monsanto Company has developed Roundup Ready corn line NK603 which is tolerant to glyphosate (the active ingredient in Roundup herbicide) at the whole plant level. Corn line NK603 contains a 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). Corn plants that demonstrate commercial level tolerance to Roundup herbicide are called Roundup Ready® (RR). The CP4 EPSPS gene from *Agrobacterium* sp. strain CP4 has been completely sequenced and encodes a 47.6-kDa protein consisting of a single polypeptide of 455 amino acids (Padgette *et al.*, 1996). The CP4 EPSPS protein is functionally similar to plant EPSPS enzymes but has a much reduced affinity for glyphosate (Padgette *et al.*, 1993). In nontransgenic plants, glyphosate binds to the plant EPSPS enzyme and blocks the biosynthesis of aromatic amino acids thereby depriving plants of these essential nutrients (Steinrucken and Amrhein, 1980; Haslam, 1993). In Roundup Ready plants, nutritional requirements for normal growth and development are met by the continued action of the glyphosate-tolerant CP4 EPSPS enzyme in the presence of glyphosate. A comprehensive safety assessment of the CP4 EPSPS protein has been described in the literature (Harrison *et al.* 1996).

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B. Purpose

The purpose of this study was to estimate levels of the CP4 EPSPS protein and to conduct compositional analyses on key corn tissues produced in 1998 U.S. field trials at two replicated sites in Illinois and Ohio (IL2, OH) and at six non-replicated sites in Iowa, Illinois, Indiana and Kansas (IA1, IA2, IA3, IL1, IN and KS). Forage and grain samples collected from corn line NK603 and the nontransgenic parental control line (LH82 x B73) were analyzed by an enzyme-linked immunosorbent assay (ELISA) to estimate the levels of CP4 EPSPS protein present in these tissues. Compositional analyses were conducted to measure proximate

(protein, fat, ash, carbohydrate, moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acid, fatty acid, vitamin E, mineral (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), phytic acid and trypsin inhibitor content of grain; and proximate, ADF and NDF content of forage. Statistical analyses were conducted for each of the replicated trials and for all trials combined, using SAS® software to determine statistically significant differences ($p < 0.05$).

III. Materials and Methods

A. Test Substance

The test substance was corn line NK603 grown in 1998 U.S. field trials (Production Plan 98-01-46-01). The test substance contains the gene encoding the CP4 EPSPS protein.

B. Control Substance

The control substance was the nontransgenic parental line (LH82 x B73) grown in 1998 U.S. field trials (Production Plan 98-01-46-01). The control line has a genetic background similar to that of the test line but lacks the gene encoding the CP4 EPSPS protein.

C. Reference Substances

The reference substances were appropriate standards used in each assay (or analytical method) as reference standards for the analytical procedures or calibration of equipment.

D. Characterization of Test and Control Substances

The identity of forage samples was based on sample handling records and CP4 EPSPS ELISA data. The identity of grain samples was based on sample handling records, CP4 EPSPS ELISA data and Southern blot analysis of genomic DNA isolated from grain.

E. Field Trials

The test and control substances were produced in 1998 U.S. field trials at six non-replicated sites and three replicated sites (Production Plan 98-01-46-01). The USDA's APHIS (Animal and Plant Health Inspection Service) requirements for the shipment, movement, environmental release and conduct of trials involving genetically-modified plants were followed in this Production Plan. A brief summary of these trials follows based on the field report (Prochaska, 1999).

The six non-replicated trials were conducted at the following sites (site code): Richland, IA (IA1); Webster City, IA (IA2); Bagley, IA (IA3); Carlyle, IL (IL1); Indianapolis, IN (IN); and, Andale, KS (KS). Three replicated trials were conducted at the following sites (site code): Jerseyville, IL (IL2); New Holland, OH (OH); and, Claude, TX (TX). Six RR corn lines (NK522, NK541, NK543, NK600, NK603 and GA21) and one control line (LH82 x B73) were planted at each site. RR corn line NK522 was dropped from commercial development

during the growing season and therefore is not included in this study. Since sufficient analytical data was already available on RR corn line GA21, it was also not included in this study. At the non-replicated sites, there were two blocks (treated and untreated) separated by a minimum buffer of 100 ft. The treated block contained six plots, one each for the six test lines, with a minimum separation distance of 45 ft between plots; the untreated block contained a single plot for the control line. Plot sizes ranged between 300-640 sq. ft. A randomized complete block design was used for the replicated sites with four blocks or replicates per site. Each block contained seven plots, one each for the seven lines, separated by a minimum distance of 10 ft. To decrease inadvertent cross-pollination between the lines, buffer rows were planted between plots. At each site, plots were planted with a row spacing of 30 in and a planting density between 21,000- 31,680 seeds/A. The genetic purity of plants was maintained by bagging the tassels and ear shoots and self-pollinating selected plants by hand in the non-replicated sites and all plants in the replicated sites.

Three applications of Roundup Ultra™ herbicide were made to plots containing transgenic lines: 1) a pre-emergence application at a target rate of 5 qt/A after planting but prior to crop emergence, 2) an early post-emergence (POE) application at a target rate of 32 fl oz/A to plants at the V4-V6 stage, and 3) a late POE application at a target rate of 32 fl oz/A to plants at the V8 or 30 in tall growth stage, whichever came first. Actual application rates were within ± 13% of the target rate. Plants showing signs of severe injury or death were excluded from sampling. Forage was collected at the late dough/early dent stage by dividing ~12 randomly selected plants into three roughly equal segments and placing them on dry ice within 10 min of collection at all sites. Ears were harvested from ~12 self-pollinated plants at normal kernel maturity (< 32% moisture), dried to a moisture between 10-20%, shelled, and the kernels pooled to provide the grain sample. Forage (on dry ice) and grain (at ambient temperature) samples were then shipped to Monsanto's facility in Chesterfield, Missouri, USA for estimation of introduced protein levels and composition analyses.

Exceptions to the sampling requirements were noted at the following sites. Due to above normal temperatures during pollination and damage due to smut disease, the amount of grain collected from the KS site was well below Production Plan requirements and insufficient for analysis. Consequently, grain samples from this site were not included in the analytical phase of the study. At the OH site, above normal temperatures during pollination and grain-fill, reduced the amount of grain to below Production Plan requirements in approximately half the samples; however, sufficient quantity and quality of grain was available from test and control lines for analysis. At the TX site, forage samples were collected from only 6 plants/plot rather than the required 12 plants/plot due to reduced germination and hail damage. Also, poor environmental conditions (high temperatures/below normal rainfall) resulted in most of the ears being infected with smut. Harvested grain was sorted to remove kernels with visual evidence of smut which caused a reduction in the amount of sample available for analysis. Statistical evaluation of the composition data showed that this trial was an outlier with respect

to the results obtained from the other field trials and not considered representative of the test and control lines. Therefore the data from this trial is not included in the final report but archived as data not reported in the study files.

F. Analytical Standards

Appropriate reagents and standards used in the CP4 ELISA assay are described below (Ledesma *et al.*, 1999). The analytical standards used for compositional analyses are described in Section III.I.

CP4 EPSPS protein standard for ELISA. The CP4 EPSPS protein standard (purity >90%; lot #5199245) was purified from an *E. coli* strain expressing the *Agrobacterium* sp. strain CP4 EPSPS gene. Aliquots of the protein standard (3.96 mg/mL) were stored at approximately -20°C in a buffer solution [50 mM Tris-HCl, pH 7.5, 50% (v/v) glycerol, 2 mM DTT, and 50 mM KCl]. The protein standard has been previously characterized (Harrison *et al.*,1993).

Antibodies. Monoclonal anti-CP4 EPSPS antibody (purity >95%; lot #6199732) was used as the capture antibody. It was purified by TSD Bioservices, Newark, DE from ascites produced from cell line 39B6.2 (Strategic Diagnostic, Newark, DE). The purified monoclonal antibody was stored at approximately 4°C, at a concentration of 3.2 mg/mL in a buffer containing 20 mM sodium phosphate, 150 mM sodium chloride and 15 ppm ProClin 300 preservative, at pH 7.2.

Goat polyclonal anti-CP4 EPSPS antibody conjugated to horse radish peroxidase (HRP) was used as the ELISA detection antibody. It was purified from goat sera HRB-G856 and HRB-G854 (Harlan Bioproducts for Science, Indianapolis, IN) using Protein G techniques (TechServ Associates, St. Louis, MO). The purified antibodies (lot #6558603-A, 8.1 mg/mL and lot 6558603-C, 7.9 mg/mL, respectively) were conjugated to HRP using a modified periodate oxidation method (GEN-PRO-077). Equal amounts of the conjugated antibodies (lot #6558603-B, 6.0 mg/mL and lot #6558603-D, 4.8 mg/mL, respectively) were pooled (lot #6558618) and aliquots were stored at approximately -20°C, at a concentration of 5.4 mg/mL, in a buffer containing 0.02 M potassium phosphate, 0.15 M sodium chloride and 0.01% thimerosal, at pH 7.3.

G. Test System

There was no test system for this study which uses analytical methods to evaluate the test and control lines. A validated CP4 EPSPS ELISA (Ledesma *et al.*, 1999) was used to estimate protein levels in forage and grain samples. Compositional analyses were performed using modifications of published methods that are currently used to evaluate the nutritional quality of corn (see Section III.I).

H. ELISA Analytical Methods

Extraction of protein from maize tissues. Corn tissues were processed according to SOPs BtM-PRO-067-01 and ES-93-ESOP-047-1, and extracts were prepared according to SOP BR-ME-0197-01. Tissues were ground to a fine powder on dry ice in a blender or a vertical cutter mixer. All tissue powders were kept on dry ice during extract preparation. Forage was extracted at a tissue-to-buffer ratio of 1:50 (w/v) with TBA (Tris-Borate with L-Ascorbic acid) buffer containing 100 mM Tris base, 100 mM sodium borate, 5 mM magnesium chloride, 0.05% (v/v) Tween 20 and 0.2% (w/v) L-ascorbic acid at pH 7.8. Grain was extracted at a tissue-to-buffer ratio of 1:100 (w/v) with PBST (Phosphate Buffered Saline with Tween 20) buffer containing 8.1 mM sodium phosphate, 138 mM sodium chloride, 1.5 mM potassium phosphate, 2.7 mM potassium chloride and 0.05% (v/v) Tween 20 at pH 7.4. Extracts were prepared using a Polytron tissue homogenizer (Brinkman, Inc., Westbury, NY) and the supernatant was removed and stored frozen at approximately -80°C until needed.

CP4 EPSPS ELISA. This assay was performed according to SOP BR-ME-0197-01. CP4 EPSPS protein levels in forage and grain extracts were estimated using a double antibody sandwich ELISA consisting of a monoclonal anti-CP4 EPSPS antibody as the capture antibody and a polyclonal anti-CP4 EPSPS conjugated to HRP as the detection antibody. A horseradish peroxidase substrate, TMB (3,3',5,5' tetramethylbenzidene), was added for color development. The CP4 EPSPS protein levels in plant tissue extracts were quantitated by comparison of the sample absorbance (OD) to the absorbance produced by a range of concentrations of the *E. coli*-produced CP4 EPSPS reference standard.

I. Compositional Analytical Methods

Forage and grain samples were prepared as described in Section H above and shipped to Covance Laboratories, Inc., Madison, Wisconsin for compositional analyses. Grain samples were analyzed for proximate (protein, fat, ash, moisture), ADF, NDF, amino acid, fatty acid, vitamin E, mineral (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc) phytic acid and trypsin inhibitor content. Forage samples were analyzed for proximate, ADF and NDF content. Carbohydrate values in forage and grain were estimated by calculation. The same methods were used for the proximate analysis of forage and grain except for the analysis of fat as described below. The analytical data generated by Covance Laboratories, Inc. was summarized in an Analytical Subreport (CHW 6103-229) which was archived with the study files.

Acid detergent fiber (ADF). This is a modified version of the method described in USDA Agricultural Handbook No. 379.8 (1970). 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 removed the fats and pigments. The lignocellulose fraction was collected on the frit and determined gravimetrically. The limit of detection of the method for

this study was 0.1% fresh weight (fw). There was no analytical reference substance for this analysis.

Amino acid composition (TAAP). This is a modified version of AOAC method 982.30 (1995). The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct hydrolysis with hydrochloric acid. The individual amino acids were then quantitated using an automated amino acid analyzer. The limit of detection of the method for this study was 0.1 mg/g. The reference standards were: Beckman K18, 2.5 µmol/mL per constituent except cystine (1.25 µmol/mL), lot no. S901670; Aldrich L-tryptophan, 99%, lot no. 12729HS; Sigma L-cysteic acid hydrate, 99.4%, lot no. 65H2658; Sigma L-methionine sulfone, 100%, lot no. 12H3349.

Ash (ASHM). This is a modified version of AOAC method 923.03 (1995). The sample was placed in an electric furnace at 550 °C and ignited to drive off volatile organics. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The limit of detection of the method for this study was 0.1% fw. There was no analytical reference substance for this analysis.

Carbohydrates (CHO). This method is described in USDA Agricultural Handbook No. 74, p 2-11 (1973). Carbohydrate values were calculated by difference using the fresh weight-derived data and the following equation:

$$\% \text{ carbohydrates} = 100\% - (\% \text{ protein} + \% \text{ fat} + \% \text{ ash} + \% \text{ moisture})$$

Fat-acid hydrolysis (FAAH). This is a modified version of AOAC methods 922.06 and 954.02 (1995). The forage sample was hydrolyzed with hydrochloric acid at elevated temperature. The fat was extracted using ether and hexane. The extracts were washed with a dilute alkali solution and filtered through a sodium sulfate column. The extract was then evaporated, dried and weighed. The limit of detection of this method for this study was 0.1% fw. There was no analytical reference substance for this analysis.

Fat-soxhlet extraction (FSOX). This is a modified version of AOAC method 960.39 (1995). The grain sample was weighed into a cellulose thimble containing sand or 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. This method was used for the grain sample analysis. The limit of detection of the method for this study was 0.1% fw.

Fatty acids (FAPM). This is a modified version of AOCS method Ce 1-62 (1981). The lipid in grain samples was extracted and saponified with 0.5 N sodium hydroxide in methanol. The

saponification mixture was methylated with 14% (v/v) 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 detection of this method for this study was 0.004% fw. The analytical reference standards (purity 100%) were: Nu Chek Prep Hazelton special prep nos 1 (lot no. JA10-I), 2 (lot no.JA10-H), 3 (lot no. F23-J), and 4 (lot no. JY30-I); and Nu Chek Prep methyl gamma linolenate (lot no. U-63M-F25-J).

Minerals/ICP emission spectrometry (ICPS). This is a modified version of AOAC methods 984.27 and 985.01 (1995) and a literature method (Dahlquist *et al.*, 1978). The sample was dried, precharred, and ashed overnight at 500° ± 50°C. The ashed sample was treated with hydrochloric acid, taken to dryness, and put into a solution of 5% (v/v) hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured by the inductively coupled plasma, with the emission of the standard solutions described below.

Mineral	Lot Numbers	Concentration (ppm)	Limit of Detection (ppm, fw)
Calcium	J5-111CA	10,000	20.0
Copper	6-137CU	1,000	0.5
Iron	6-172FE	1,000	2.00
Magnesium	K5-67MG	10,000	20.0
Manganese	6-55MN	1,000	0.3
Phosphorus	15-75P	10,000	20.0
Potassium	L5-149K	10,000	100.0
Sodium	L5-80NA	10,000	100.0
Zinc	6-171ZN	1,000	0.4

Moisture (M100). This is a modified version of AOAC methods 926.08 and 925.09 (1995). The sample was 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 detection of this method for this study was 0.1% fw. There was no analytical reference substance for this analysis.

Neutral detergent fiber, enzyme method (NDFE). This is a modified version of AACC method 32.20 (1983) and the method listed in USDA Agricultural Handbook No. 379 (1970). The sample was placed in a fritted vessel and washed with a boiling detergent solution that dissolved the protein, carbohydrate, enzyme and ash. An acetone wash removed the fats and pigments. The hemicellulose, cellulose and lignin fractions were collected on the frit and

determined gravimetrically. The limit of detection of this method for this study was 0.1% fw. There was no analytical reference substance for this analysis.

Phytic acid (VCXX). This a modification of two literature methods (Lehrfeld 1989, 1994). The sample was extracted using ultrasonication. Purification and concentration was done on a silica based anion exchange (SAX) column. Sample analysis was done on a macroporous polymer HPLC column PRP-1, 5µm (150 x 4.1) and a refractive index detector. The limit of quantitation for this study was between 0.05 and 0.08% fw. The reference substance for this assay was Aldrich phytic acid, dodecasodium salt hydrate, 99%, lot no. 13529MS.

Protein (PGEN). This is a modified version of AOAC methods 955.04 and 979.09 (1995) and literature methods (Bradstreet, 1965; Kalthoff and Sandell, 1948). Protein and other nitrogenous compounds in the sample were reduced to ammonia by digesting 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 protein using the factor 6.25. The limit of detection of this method for this study was 0.1% fw. There was no analytical reference substance for this analysis.

Trypsin inhibitor (MIXX). This is a modified version of AOCS method Ba 12-75 (1997). Trypsin inhibitor activity in the sample was determined by suspending the ground, defatted sample in dilute sodium hydroxide solution. An appropriate dilution of the suspension was made, and an increasing series of aliquots of the diluted suspension was mixed with trypsin and benzoyl-DL-arginine-p-nitroanilide. After 10 minutes, the action of the trypsin was stopped by the addition of acetic acid. The diluted suspension mixture was filtered or centrifuged and the absorbance of each filtered solution was measured at 410 nm. Trypsin inhibitor activity was calculated from the change in absorbance values due to the aliquot volume. The limit of detection for this study was 1.0 TIU/mg fw.

Vitamin E (EFD2). This a modification of a literature method (Cort *et al.*, 1983). The sample was saponified to break down any fat and release any vitamin E. The saponified mixture was extracted with ethyl ether and then quantitated directly by high-performance liquid chromatography on a silica column. The limit of quantitation for this study was between 0.001 and 0.002 mg/g fw. The reference substance for this assay was USP alpha tocopherol, 100%, lot number L1.

J. Control of Bias

Corn tissues were ground thoroughly and mixed before extraction to minimize tissue bias. During the validation of the ELISA methods used in this study, the accuracy of the system was evaluated and the method optimized to minimize assay bias. Accuracy is defined by two components: extraction efficiency and recovery of purified protein standard spiked into the

control matrix. CP4 EPSPS protein levels reported in this study were corrected for assay bias (see Table 1).

K. Data Reduction and Statistical Analysis

Data reduction to estimate CP4 EPSPS protein levels were conducted using Soft max Pro software (version 2.4.1) available from Molecular Devices (Sunnyvale, CA). Microsoft Excel™ was used to transform the CP4 EPSPS ELISA data for the calculation of means and standard deviations of protein levels.

Statistical analyses of the composition data was conducted by Certus International, Inc., Chesterfield, MO 63017, USA. Analytes that had >50% of values at or below the LOD of the assay were excluded from statistical analysis.

Statistical analyses were conducted using a mixed model analysis of variance for three sets of comparisons: analyses for each of the two replicated trials and for a combination of trials at different field sites. Individual replicated trial analyses used the model:

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

where U = overall mean, T_i = line effect, B_j = random block effect, and e_{ij} = residual error.

Combined trial analyses used the model:

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

where U = overall mean, T_i = line effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by line interaction effect, and e_{ijk} = residual error. In these analyses, corn line NK603 was compared to the nontransgenic control line LH82 x B73. Analyses for corn lines NK541, NK543 and NK600 were not reported since these lines were dropped for commercial reasons.

SAS® software was used to generate all summary statistics and perform all analyses (SAS Institute, 1989, 1990, 1996). Report tables present p-values from SAS® as either <0.001 or the actual value truncated to three decimal places.

L. Protocol Amendments

Protocol Amendment #1. Vitamin E was added to the list of components to be analyzed in grain.

Protocol Amendment #2. Protein level determination was amended by requiring CP4 ELISA analysis of individual replicate sample/line for the replicated sites.

Protocol Amendment #3. The facility was changed from Monsanto to Certus International, Inc., for the conduct of statistical analysis.

Protocol Amendment #4. Lines NK541, NK543 and NK600 were deleted from the study for commercial reasons. Data from the TX site were excluded from the study because it was not representative of the test and control lines. Grain from the KS site were excluded from analysis due to poor yield and poor quality. Corrections were made to the city and county name for some field sites.

Protocol Amendment #5. During a review of the analysis of variance for this study it was discovered that the SAS program had not correctly accounted for the site variability in the combined site analysis. The correction of this error resulted in the proper incorporation of site-to-site variability in the combined site statistical analysis. All other statistical analyses remained the same.

IV. Results and Discussion

A. Characterization of Test and Control Substances

All forage samples produced from corn line NK603 and the control line were correctly identified based on sampling handling records and ELISA data. The range of CP4 EPSPS levels in forage for corn line NK603 line did not overlap with any of the other test lines which provided a valid method for their identity. The levels of CP4 EPSPS protein in all control samples were below the limit of quantitation (LOQ) of the method (see Section IV.B below).

All grain samples produced from corn line NK603 and the control line were correctly identified based on sample handling records, ELISA and Southern blot analysis data. CP4 EPSPS protein levels were above the LOQ of the method in all NK603 samples and below the LOQ of the method in all control samples. Southern blot analysis for the presence of the CTP2-CP4 EPSPS gene gave the expected fingerprint of bands for all seven NK603 grain samples tested, while no hybridizing bands were found for the six control samples tested in this assay.

B. CP4 EPSPS Protein Levels in Corn Tissues

Table 1 summarizes the CP4 EPSPS protein levels determined in NK603 corn forage and grain samples, corrected for assay bias. Mean CP4 EPSPS protein levels in NK603 forage were comparable for the non-replicated sites (25.5 µg/g fwt) and replicated sites (25.9 µg/g fwt). CP4 EPSPS protein levels in control forage were below the LOQ of the assay (< 0.05

µg/g fwt). Mean CP4 EPSPS protein levels in NK603 grain were comparable for the non-replicated sites (11.0 µg/g fwt) and replicated sites (10.6 µg/g fwt). CP4 EPSPS protein levels in control grain were below the LOQ of the assay (< 0.09 µg/g fwt). Therefore, it is concluded that the CP4 EPSPS protein introduced into corn line NK603 is expressed at approximately the same levels either within site or across geographically dispersed sites.

C. Compositional Analyses and Statistical Evaluation

The compositional analysis data and statistical evaluation are summarized in Tables 2 to 8. Statistical analyses of the data were conducted as described in Section III.K. Component values are expressed as follows: amino acids as % total amino acids; proximates (except moisture), ADF, NDF, magnesium, phytic acid and potassium as % dry weight (dw).; moisture as % fresh wt.; fatty acids as % total fatty acids; copper, iron, manganese and zinc as mg/kg dw; vitamin E as mg/g dw; and trypsin inhibitor in TIU (trypsin inhibitor units)/mg dw. The following components are not listed in Tables 2-8 since they had >50% of values below the LOD of the assay: sodium, 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, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid.

Fifty-one different compositional components were evaluated for corn line NK603 as part of the safety and nutritional assessment of this product. The values for all the compositional components assessed were either within the range observed for nontransgenic commercial corn lines, published literature ranges (Jugenheimer, 1976; Watson, 1982; Watson, 1987) or previously reported ranges for nontransgenic corn varieties (Sanders and Patzer, 1995; Sanders *et al.*, 1996a,b; 1997a,b). Data were developed and statistical analyses conducted for three sets of comparisons: analyses for each of two replicated trials and for a combination of trials at different field sites. Therefore, a total of 153 comparisons were made, 51 comparisons for each of these three statistical analyses.

Statistical evaluation showed that there were no statistically significant differences in 135 of the 153 comparisons made between corn line NK603 and the control line. Statistically significant differences were noted for carbohydrates, protein and moisture in forage, and for arginine, cystine, phenylalanine, 16:0 palmitic acid (twice), 18:0 stearic acid (three times), 18:1 oleic acid (twice), 20:1 eicosenoic acid, calcium, magnesium, phosphorus and moisture in grain (see Table 2). Of the 18 comparisons found to be statistically significantly different, 7–8 (0.05 x 153) are expected to be false positives based on chance alone. The two differences noted for moisture are not of nutritional significance and therefore not considered further. Only one of the statistically significant differences was consistently observed for each of the two replicated trial comparisons and the comparisons across sites. Differences which were observed for only one or two of these comparisons, and not consistently across all three comparisons, are not considered biologically meaningful or relevant. Furthermore, all of these

differences were well within reported ranges for corn. The only component for which statistically significant differences were observed across all three statistical evaluations was 18:0 stearic acid in grain. The absolute magnitude of the differences as a percent of the corresponding control mean value for stearic acid ranged between 3.90-5.41%. These values are well within the range of natural variability and the published ranges for stearic acid in corn grain, and therefore, these differences are not considered biologically relevant. Grain and forage from corn line NK603 are considered compositionally equivalent to that of conventional corn.

V. Conclusions

ELISA results showed that mean CP4 EPSPS protein levels in corn line NK603 were comparable for non-replicated sites and replicated sites in forage as well as in grain. Therefore, it is concluded that the CP4 EPSPS protein introduced into corn line NK603 is expressed at approximately the same levels either within site or across geographically dispersed sites.

Statistical evaluation showed that there were no statistically significant differences in 135 of the 153 comparisons made between corn line NK603 and the control line. Only one of the statistically significant differences was consistently observed for each of the two replicated trial comparisons and the comparisons across sites. Differences which were observed for only one or two of these comparisons, and not consistently across all three comparisons, are not considered biologically meaningful or relevant. Furthermore, all of these differences were well within reported ranges for corn. The only component for which statistically significant differences were observed across all three statistical evaluations was 18:0 stearic acid in grain. The absolute magnitude of the differences as a percent of the corresponding control mean value for stearic acid ranged between 3.90-5.41%. These values are well within the range of natural variability and the published ranges for stearic acid in corn grain, and therefore, these differences are not considered biologically relevant. Grain and forage from corn line NK603 are considered compositionally equivalent to that of conventional corn.

VI. Acknowledgments

The authors would like to acknowledge Roy Fuchs, Kent Croon and Kevin Glenn for critical review of this report. We thank Environmental Sciences sample preparation group for tissue processing, Henry Bauer for sample shipment, John Herbert for extract preparations, Margaret A. Nemeth for review of the statistical results and Susan Riordan for facilitating statistical analysis of the composition data.

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Table 1. CP4 EPSPS Protein Levels in RR Corn Line NK603 Tissues Produced in 1998 U.S. Field Trials

Trial	Parameter	Forage[a,c] (µg/g fwt)	Grain[b,c] (µg/g fwt)
Non-replicated	mean	25.5	11.0
	range	18.0 - 31.2	6.9-15.6
	SD*	4.5	3.2
Replicated	mean	25.9	10.6
	range	25.7-26.1	9.8-11.3
	SD*	0.3	1.0
All trials	mean	25.6	10.9
	range	18.0 - 31.2	6.9-15.6
	SD*	3.8	2.6

*SD = Standard Deviation.
[a]LOQ = 0.05 µg/g fwt.
[b]LOQ = 0.09 µg/g fwt.
[c]Values for all control samples below the LOQ of the assay.

Table 2. Summary of Statistically Significant Differences

Tissue/ Component[a]	p < 0.05[b]			Mean NK603[b]			Mean Control[b]			Mean difference[b] (NK603 minus control)			Mean difference[c] % of control value		
	IL2	OH	All trials	IL2	OH	All trials	IL2	OH	All trials	IL2	OH	All trials	IL2	OH	All trials
Forage															
Carbohydrate	-	0.049	-	-	87.94	-	-	89.25	-	-	-1.31	-	-	-1.47	-
Protein	-	0.047	-	-	7.86	-	-	6.46	-	-	1.4	-	-	21.67	-
Moisture[d]	0.011	-	-												
Grain															
Arginine	0.037	-	-	4.31	-	-	4.55	-	-	-0.24	-	-	-5.27	-	-
Cystine	0.016	-	-	2.14	-	-	1.88	-	-	0.26	-	-	13.83	-	-
Phenylalanine	-	0.033	-	-	5.43		-	5.31		-	0.11		-	2.07	
16:0 Palmitic acid	-	0.007	<0.001	-	8.94	9.13	-	8.6	8.89	-	0.34	0.24	-	3.95	2.70
18:0 Stearic acid	0.027	0.001	0.001	2.03	1.95	1.92	1.95	1.85	1.83	0.076	0.1	0.094	3.90	5.41	5.14
18:1 Oleic acid	0.003	-	0.007	22.45	-	22.4	23.38	-	23.08	-0.93	-	-0.68	-3.98	-	-2.95
20:1 Eicosenoic acid	-	0.049	-	-	0.3		-	0.33		-	-0.026		-	-7.88	
Calcium	<0.001	-	-	0.004	-	-	0.0034	-	-	0.00059	-	-	17.35	-	-
Magnesium	0.028	-	-	0.11	-	-	0.11	-	-	0.0058	-	-	5.27	-	-
Phosphorus	0.007	-	-	0.35	-	-	0.33	-	-	0.019	-	-	5.76	-	-
Moisture[d]	0.005	-	-												

[a] Carbohydrate, protein, calcium, potassium and magnesium as % dw; amino acids as % of total; fatty acids as % of total.

[b] Data obtained from Tables 3-8.

[c] Calculated values.

[d] Only p values listed as moisture differences not considered nutritionally significant (see text). The differences can be found in Tables 3 to 8.

Amendment 1

Table 3. Replicated Trial (Illinois): Fiber and Proximate Content of Forage and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Commercial[e] (Range)	Reported[f] (Range)
Ash (% dw)	4.49 ± 0.28 (3.59 - 4.88)	4.62 ± 0.28 (3.85 - 5.15)	-0.13 ± 0.40 (-0.27 - 0.12)	0.744	-1.00, 0.73	(2.03 - 7.49)	(2.9 - 5.1)
Carbohydrates (% dw)	83.85 ± 0.70 (82.68 - 85.15)	84.56 ± 0.70 (83.71 - 86.10)	-0.71 ± 0.94 (-1.57 - -0.15)	0.465	-2.77, 1.35	(81.5 - 88.9)	(84.6 - 89.1)
ADF (% dw)	28.74 ± 0.91 (26.48 - 33.52)	27.65 ± 0.91 (25.55 - 29.01)	1.09 ± 1.28 (-1.33 - 4.51)	0.412	-1.71, 3.89	(17.6 - 34.5)	(21.4 - 29.2)
NDF (% dw)	41.94 ± 1.09 (39.39 - 46.00)	39.51 ± 1.09 (35.44 - 42.34)	2.42 ± 1.54 (-1.00 - 10.56)	0.140	-0.93, 5.77	(29.6 - 50.7)	(39.9 - 46.6)
Moisture (% fw)	74.40 ± 0.39 (73.60 - 75.00)	72.75 ± 0.39 (71.70 - 73.70)	1.65 ± 0.56 (1.30 - 2.00)	0.011	0.43, 2.87	(47.0 - 78.8)	(68.7 - 73.5)
Protein (% dw)	8.80 ± 0.34 (8.56 - 8.98)	8.21 ± 0.34 (7.87 - 8.69)	0.59 ± 0.42 (0.29 - 0.81)	0.182	-0.32, 1.50	(4.93 - 11.0)	(4.8 - 8.4)
Total fat (% dw)	2.86 ± 0.27 (2.58 - 3.50)	2.60 ± 0.27 (2.10 - 3.42)	0.25 ± 0.38 (-0.77 - 1.41)	0.510	-0.56, 1.07	(0.79 - 3.64)	(1.4 - 2.1)

[a]ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt.
[b]The mean of four replicate values.
[c]S.E. = standard error of the mean.
[d]C.I. = confidence interval.
[e]The range of sample values across commercial lines grown in 1998 (Sidhu *et al.*, 1999).
[f]Range for two control lines analysed in Monsanto Company trials conducted in 1994 and 1995 (Sanders *et al.*, 1996b; 1997a).

Table 4. Replicated Trial (Illinois): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Amino acids (% of total)								
Alanine	7.90 ± 0.037 (7.85 - 7.98)	7.98 ± 0.037 (7.90 - 8.05)	-0.077 ± 0.048 (-0.13 - -0.042)	0.137	-0.18, 0.028	(7.1 - 8.2)	(6.4-9.9)	(7.3-8.8)
Arginine	4.31 ± 0.073 (4.24 - 4.47)	4.55 ± 0.073 (4.35 - 4.63)	-0.24 ± 0.10 (-0.39 - 0.13)	0.037	-0.47, -0.016	(4.0 - 5.5)	(2.9-5.9)	(3.6-5.0)
Aspartic acid	6.41 ± 0.040 (6.29 - 6.48)	6.40 ± 0.040 (6.29 - 6.51)	0.011 ± 0.057 (-0.17 - 0.18)	0.844	-0.11, 0.14	(6.3 - 7.4)	(5.8-7.2)	(6.3-7.5)
Cystine	2.14 ± 0.071 (2.05 - 2.27)	1.88 ± 0.071 (1.63 - 2.01)	0.26 ± 0.094 (0.094 - 0.52)	0.016	0.057, 0.47	(1.8 - 2.9)	(1.2-1.6)	(1.8-2.7)
Glutamic acid	19.48 ± 0.073 (19.16 - 19.67)	19.32 ± 0.073 (19.19 - 19.43)	0.16 ± 0.10 (-0.19 - 0.34)	0.149	-0.066, 0.38	(17.4 - 20.1)	(12.4-19.6)	(19.5-22.8)
Glycine	3.64 ± 0.053 (3.52 - 3.74)	3.74 ± 0.053 (3.61 - 3.86)	-0.10 ± 0.075 (-0.26 - 0.13)	0.206	-0.26, 0.063	(3.4 - 4.6)	(2.6-4.7)	(3.2-4.2)
Histidine	2.77 ± 0.029 (2.72 - 2.81)	2.79 ± 0.029 (2.76 - 2.88)	-0.022 ± 0.041 (-0.094 - 0.049)	0.597	-0.11, 0.067	(2.6 - 3.4)	(2.0-2.8)	(2.8-3.3)
Isoleucine	3.86 ± 0.043 (3.81 - 3.91)	3.81 ± 0.043 (3.73 - 3.91)	0.051 ± 0.061 (-0.053 - 0.099)	0.415	-0.082, 0.18	(3.0 - 4.1)	(2.6-4.0)	(3.2-4.3)
Leucine	13.90 ± 0.094 (13.63 - 14.05)	13.75 ± 0.094 (13.59 - 13.91)	0.15 ± 0.13 (-0.28 - 0.46)	0.295	-0.14, 0.44	(11.3 - 14.4)	(7.8-15.2)	(12.6-15.8)

(continued over)

Table 4. Replicated Trial (Illinois): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Lysine	2.79 ± 0.059 (2.64 - 2.96)	2.86 ± 0.059 (2.72 - 3.00)	-0.068 ± 0.083 (-0.22 - 0.24)	0.427	-0.25, 0.11	(2.6 - 3.9)	(2.0-3.8)	(2.6-3.5)
Methionine	2.13 ± 0.056 (2.08 - 2.16)	1.97 ± 0.056 (1.74 - 2.14)	0.15 ± 0.078 (-0.0037 - 0.42)	0.075	-0.018, 0.32	(1.6 - 2.9)	(1.0-2.1)	(1.3-2.6)
Phenylalanine	5.28 ± 0.026 (5.20 - 5.33)	5.22 ± 0.026 (5.15 - 5.27)	0.056 ± 0.034 (-0.037 - 0.13)	0.120	-0.017, 0.13	(4.7 - 5.5)	(2.9-5.7)	(5.0-6.1)
Proline	8.93 ± 0.067 (8.60 - 9.10)	8.90 ± 0.067 (8.84 - 8.96)	0.030 ± 0.095 (-0.35 - 0.25)	0.755	-0.18, 0.24	(8.0 - 9.9)	(6.6-10.3)	(8.7-10.1)
Serine	4.77 ± 0.034 (4.72 - 4.84)	4.87 ± 0.034 (4.84 - 4.91)	-0.098 ± 0.046 (-0.18 - -0.040)	0.056	-0.20, 0.0031	(3.5 - 5.5)	(4.2-5.5)	(4.9-6.0)
Threonine	3.40 ± 0.026 (3.36 - 3.46)	3.38 ± 0.026 (3.29 - 3.47)	0.025 ± 0.035 (-0.11 - 0.099)	0.494	-0.051, 0.10	(3.1 - 4.0)	(2.9-3.9)	(3.3-4.2)
Tryptophan	0.54 ± 0.016 (0.47 - 0.56)	0.58 ± 0.016 (0.56 - 0.59)	-0.042 ± 0.022 (-0.11 - 0.0014)	0.087	-0.090, 0.0072	(0.4 - 0.8)	(0.5-1.2)	(0.4-1.0)
Tyrosine	2.94 ± 0.25 (2.46 - 3.40)	3.18 ± 0.25 (2.43 - 3.49)	-0.24 ± 0.35 (-0.98 - 0.086)	0.495	-1.00, 0.51	(2.1 - 4.0)	(2.9-4.7)	(3.7-4.3)
Valine	4.82 ± 0.042 (4.76 - 4.85)	4.82 ± 0.042 (4.73 - 4.94)	-0.00096 ± 0.059 (-0.094 - 0.092)	0.987	-0.13, 0.13	(3.9 - 5.5)	(2.1-5.2)	(4.2-5.3)

(continued over)

Table 4. Replicated Trial (Illinois): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Fatty acids (% of total)								
16:0 palmitic acid	9.50 ± 0.067 (9.42 - 9.57)	9.32 ± 0.067 (9.23 - 9.44)	0.18 ± 0.094 (-0.022 - 0.34)	0.085	-0.029, 0.38	(8.8 - 13.8)	(7-19)	(9.9-12.0)
18:0 stearic acid	2.03 ± 0.021 (1.97 - 2.06)	1.95 ± 0.021 (1.92 - 1.98)	0.076 ± 0.030 (0.032 - 0.14)	0.027	0.010, 0.14	(1.4 - 2.6)	(1-3)	(1.4-2.2)
18:1 oleic acid	22.45 ± 0.18 (22.04 - 22.62)	23.38 ± 0.18 (23.09 - 23.78)	-0.93 ± 0.25 (-1.23 - -0.47)	0.003	-1.48, -0.38	(20.7 - 37.7)	(20-46)	(20.6-27.5)
18:2 linoleic acid	64.09 ± 0.24 (63.81 - 64.64)	63.41 ± 0.24 (63.07 - 63.74)	0.67 ± 0.33 (0.070 - 1.13)	0.066	-0.052, 1.40	(48.0 - 66.1)	(35-70)	(55.9-66.1)
18:3 linolenic acid	1.09 ± 0.011 (1.08 - 1.11)	1.09 ± 0.011 (1.07 - 1.11)	0.0019 ± 0.015 (-0.026 - 0.018)	0.901	-0.031, 0.035	(0.9 - 1.5)	(0.8-2)	(0.8-1.1)
20:0 arachidic acid	0.38 ± 0.0057 (0.38 - 0.39)	0.38 ± 0.0057 (0.38 - 0.40)	-0.00059 ± 0.0080 (-0.013 - 0.016)	0.942	-0.018, 0.017	(0.3 - 0.6)	(0.1-2)	(0.3-0.5)
20:1 eicosenoic acid	0.29 ± 0.0062 (0.28 - 0.29)	0.29 ± 0.0062 (0.27 - 0.30)	0.00007 ± 0.0088 (-0.020 - 0.019)	0.993	-0.019, 0.019	(0.2 - 0.4)	(na)	(0.2-0.3)
22:0 behenic acid	0.17 ± 0.0053 (0.16 - 0.17)	0.17 ± 0.0053 (0.16 - 0.18)	0.00004 ± 0.0076 (-0.010 - 0.011)	0.996	-0.016, 0.017	(0.1 - 0.3)	(na)	(0.1-0.3)

(continued over)

Table 4. Replicated Trial (Illinois): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Minerals								
Calcium (%)	0.0040 ± 0.00009 (0.0037 - 0.0043)	0.0034 ± 0.00009 (0.0033 - 0.0036)	0.00059 ± 0.00011 (0.00034 - 0.00091)	<0.001	0.00034, 0.00083	(0.003 - 0.009)	(0.01-0.1)	(0.003-0.006)
Copper (mg/kg dw)	2.20 ± 0.097 (2.06 - 2.37)	2.22 ± 0.097 (2.01 - 2.33)	-0.014 ± 0.14 (-0.24 - 0.36)	0.922	-0.31, 0.28	(0.9 - 2.8)	(0.9-10)	(na)
Iron (mg/kg dw)	25.68 ± 0.48 (25.39 - 25.94)	24.91 ± 0.48 (23.59 - 26.62)	0.77 ± 0.68 (-1.23 - 2.18)	0.281	-0.72, 2.25	(11 - 49)	(1-100)	(na)
Magnesium (%)	0.11 ± 0.0018 (0.11 - 0.12)	0.11 ± 0.0018 (0.11 - 0.11)	0.0058 ± 0.0023 (0.0033 - 0.0074)	0.028	0.00074, 0.011	(0.08 - 0.2)	(0.09-1.0)	(na)
Manganese (mg/kg dw)	5.34 ± 0.091 (5.03 - 5.77)	5.53 ± 0.091 (5.38 - 5.63)	-0.19 ± 0.13 (-0.56 - 0.25)	0.173	-0.47, 0.094	(2.6 - 7.8)	(0.7-54)	(na)
Phosphorus (%)	0.35 ± 0.0041 (0.34 - 0.36)	0.33 ± 0.0041 (0.32 - 0.34)	0.019 ± 0.0058 (0.010 - 0.025)	0.007	0.0062, 0.032	(0.24 - 0.43)	(0.26-0.75)	(0.31-0.36)
Potassium (%)	0.36 ± 0.0041 (0.35 - 0.36)	0.35 ± 0.0041 (0.34 - 0.36)	0.011 ± 0.0058 (0.0055 - 0.016)	0.086	-0.0018, 0.024	(0.29 - 0.53)	(0.32-0.72)	(na)
Zinc (mg/kg dw)	30.78 ± 0.72 (29.18 - 32.07)	29.96 ± 0.72 (27.93 - 31.37)	0.81 ± 1.01 (-1.24 - 4.14)	0.439	-1.40, 3.02	(15 - 33)	(12-30)	(na)

(continued over)

Table 4. Replicated Trial (Illinois): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Fiber and Proximates								
Ash (% dw)	1.40 ± 0.035 (1.29 - 1.47)	1.42 ± 0.035 (1.32 - 1.51)	-0.020 ± 0.050 (-0.15 - 0.14)	0.700	-0.13, 0.089	(0.8 - 1.8)	(1.1-3.9)	(1.2-1.8)
Carbohydrates (% dw)	83.84 ± 0.16 (83.63 - 84.06)	83.46 ± 0.16 (83.22 - 83.70)	0.38 ± 0.23 (-0.074 - 0.84)	0.117	-0.11, 0.87	(83.1 - 89.6)	(na)	(na)
ADF (% dw)	3.93 ± 0.20 (3.48 - 4.22)	3.98 ± 0.20 (3.78 - 4.19)	-0.053 ± 0.27 (-0.71 - 0.31)	0.846	-0.64, 0.53	(2.3 - 5.7)	(3.3 - 4.3)	(3.1 - 5.3)
NDF (% dw)	10.30 ± 1.15 (9.34 - 12.06)	10.13 ± 1.15 (9.06 - 11.38)	0.18 ± 1.23 (-1.83 - 2.09)	0.888	-2.50, 2.85	(8.2 - 16.1)	(8.3-11.9)	(9.6 - 15.3)
Moisture (% fw)	12.28 ± 0.37 (11.00 - 13.30)	14.00 ± 0.37 (13.40 - 14.80)	-1.73 ± 0.52 (-2.60 - -0.90)	0.005	-2.85, -0.60	(6.1 - 15.6)	(7-23)	(9.4 - 15.8)
Total fat (%)	3.63 ± 0.11 (3.39 - 3.87)	3.91 ± 0.11 (3.81 - 4.13)	-0.28 ± 0.16 (-0.42 - 0.046)	0.115	-0.63, 0.079	(1.74 - 4.31)	(3.1-5.7, 2.9-6.1)	(2.4-4.2)
Protein (% dw)	11.13 ± 0.13 (10.84 - 11.37)	11.21 ± 0.13 (11.02 - 11.60)	-0.084 ± 0.18 (-0.34 - 0.35)	0.655	-0.48, 0.32	(6.7 - 13.4)	(6.0 - 12.0, 9.7 - 16.1)	(9.0 - 13.6)

(continued over)

Table 4. Replicated Trial (Illinois): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Miscellaneous								
Phytic Acid (%)	0.90 ± 0.047 (0.70 - 1.05)	0.92 ± 0.047 (0.83 - 1.02)	-0.022 ± 0.058 (-0.23 - 0.15)	0.715	-0.15, 0.11	(0.5 - 1.3)	(to 0.9%)	(na)
Trypsin Inhibitor (TIU/mg dw)	3.76 ± 0.55 (2.66 - 5.08)	3.29 ± 0.55 (2.43 - 5.14)	0.47 ± 0.78 (-2.15 - 2.07)	0.559	-1.24, 2.18	(3.40 - 7.18)	(na)	(na)
Vitamin E (mg/g dw)	0.0095 ± 0.00027 (0.0089 - 0.010)	0.0093 ± 0.00027 (0.0087 - 0.010)	0.00019 ± 0.00035 (-0.00083 - 0.0013)	0.592	-0.00057, 0.00095	(0.006 - 0.022)	(0.017- 0.047)	(0.008- 0.012)

[a]ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt; TIU = trypsin inhibitor units.
[b]The mean of four replicate values.
[c]S.E. = standard error of the mean.
[d]C.I. = confidence interval.
[e]Comm. = commercial. The range of sample values for commercial lines grown in 1998 (Sidhu *et al.*, 1999).
[f]Lit. = literature. For amino and fatty acids, Watson, 1982; for all other components, Watson, 1987; protein and fat second values from Jugenheimer, 1976.
[g]Rpt. = reported. For amino and fatty acids, range for five control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a,b; 1997a,b).
[h]For ash, moisture and total fat, range for five control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a,b; 1997a,b).
[i]For ADF and NDF, range for three control lines analysed in Monsanto trials conducted between 1994 and 1995 (Sanders *et al.*, 1996b; 1997a,b).
[j]For calcium and phosphorus, range for three control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a; 1997b).

Table 5. Replicated Trial (Ohio): Fiber and Proximate Content of Forage and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Commercial[e] (Range)	Reported[f] (Range)
Ash (% dw)	3.36 ± 0.19 (2.88 - 3.67)	3.29 ± 0.19 (2.89 - 3.66)	0.062 ± 0.26 (-0.46 - 0.40)	0.814	-0.51, 0.63	(2.03 - 7.49)	(2.9 - 5.1)
Carbohydrates (% dw)	87.94 ± 0.43 (87.23 - 89.18)	89.25 ± 0.43 (87.95 - 89.97)	-1.31 ± 0.60 (-2.41 - -0.093)	0.049	-2.61, -0.0052	(81.5 - 88.9)	(84.6 - 89.1)
ADF (% dw)	28.91 ± 0.84 (27.45 - 29.48)	28.10 ± 0.84 (25.07 - 30.58)	0.81 ± 1.19 (-3.13 - 4.20)	0.509	-1.78, 3.39	(17.6 - 34.5)	(21.4 - 29.2)
NDF (% dw)	47.43 ± 1.36 (46.53 - 48.14)	48.48 ± 1.36 (44.88 - 51.10)	-1.05 ± 1.71 (-3.13 - 3.14)	0.549	-4.77, 2.67	(29.6 - 50.7)	(39.9 - 46.6)
Moisture (% fw)	65.88 ± 0.88 (63.20 - 67.80)	63.88 ± 0.88 (61.90 - 66.10)	2.00 ± 1.25 (-0.70 - 5.20)	0.134	-0.72, 4.72	(47.0 - 78.8)	(68.7 - 73.5)
Protein (% dw)	7.86 ± 0.49 (6.04 - 8.51)	6.46 ± 0.49 (5.66 - 7.64)	1.40 ± 0.63 (0.38 - 2.20)	0.047	0.020, 2.78	(4.9 - 11.0)	(4.8 - 8.4)
Total fat (% dw)	0.84 ± 0.12 (0.69 - 1.11)	0.99 ± 0.12 (0.61 - 1.52)	-0.15 ± 0.17 (-0.77 - 0.21)	0.387	-0.52, 0.22	(0.79 - 3.64)	(1.4 - 2.1)

[a]ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt.
[b]The mean of four replicate values.
[c]S.E. = standard error of the mean.
[d]C.I. = confidence interval.
[e]The range of sample values across commercial lines grown in 1998 (Sidhu *et al.*, 1999).
[f]Range for two control lines analyzed in Monsanto Company trials conducted in 1994 and 1995 (Sanders *et al.*, 1996b; 1997a).

Table 6. Replicated Trial (Ohio): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Amino acids (% of total)								
Alanine	8.17 ± 0.046 (8.07 - 8.22)	8.09 ± 0.046 (8.01 - 8.17)	0.082 ± 0.065 (0.039 - 0.17)	0.234	-0.061, 0.22	(7.1 - 8.2)	(6.4-9.9)	(7.3-8.8)
Arginine	3.88 ± 0.086 (3.80 - 3.94)	3.96 ± 0.086 (3.90 - 4.04)	-0.081 ± 0.12 (-0.099 - -0.051)	0.517	-0.35, 0.18	(4.0 - 5.5)	(2.9-5.9)	(3.6-5.0)
Aspartic acid	6.49 ± 0.068 (6.43 - 6.62)	6.43 ± 0.068 (6.34 - 6.56)	0.055 ± 0.096 (-0.033 - 0.10)	0.580	-0.15, 0.26	(6.3 - 7.4)	(5.8-7.2)	(6.3-7.5)
Cystine	1.75 ± 0.058 (1.69 - 1.79)	1.87 ± 0.058 (1.68 - 2.15)	-0.12 ± 0.074 (-0.38 - 0.088)	0.127	-0.28, 0.040	(1.8 - 2.9)	(1.2-1.6)	(1.8-2.7)
Glutamic acid	20.27 ± 0.10 (20.15 - 20.47)	20.23 ± 0.10 (20.11 - 20.41)	0.043 ± 0.15 (-0.085 - 0.10)	0.776	-0.28, 0.36	(17.4 - 20.1)	(12.4-19.6)	(19.5-22.8)
Glycine	3.29 ± 0.065 (3.22 - 3.37)	3.37 ± 0.065 (3.30 - 3.51)	-0.079 ± 0.092 (-0.14 - 0.033)	0.402	-0.28, 0.12	(3.4 - 4.6)	(2.6-4.7)	(3.2-4.2)
Histidine	2.55 ± 0.033 (2.45 - 2.62)	2.62 ± 0.033 (2.56 - 2.70)	-0.071 ± 0.041 (-0.11 - -0.00035)	0.107	-0.16, 0.018	(2.6 - 3.4)	(2.0-2.8)	(2.8-3.3)
Isoleucine	3.93 ± 0.047 (3.84 - 4.06)	3.86 ± 0.047 (3.81 - 3.93)	0.070 ± 0.066 (-0.019 - 0.13)	0.312	-0.074, 0.21	(3.0 - 4.1)	(2.6-4.0)	(3.2-4.3)
Leucine	14.69 ± 0.13 (14.58 - 14.79)	14.45 ± 0.13 (14.39 - 14.50)	0.24 ± 0.19 (0.19 - 0.29)	0.227	-0.17, 0.64	(11.3 - 14.4)	(7.8-15.2)	(12.6-15.8)

(continued over)

Table 6. Replicated Trial (Ohio): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Lysine	2.49 ± 0.079 (2.42 - 2.55)	2.49 ± 0.079 (2.41 - 2.65)	-0.0013 ± 0.11 (-0.11 - 0.093)	0.990	-0.25, 0.24	(2.6 - 3.9)	(2.0-3.8)	(2.6-3.5)
Methionine	1.83 ± 0.050 (1.76 - 1.86)	1.96 ± 0.050 (1.83 - 2.08)	-0.13 ± 0.062 (-0.22 - 0.015)	0.056	-0.27, 0.0045	(1.6 - 2.9)	(1.0-2.1)	(1.3-2.6)
Phenylalanine	5.43 ± 0.034 (5.35 - 5.52)	5.31 ± 0.034 (5.28 - 5.36)	0.11 ± 0.047 (0.044 - 0.16)	0.033	0.010, 0.22	(4.7 - 5.5)	(2.9-5.7)	(5.0-6.1)
Proline	8.58 ± 0.077 (8.44 - 8.69)	8.68 ± 0.077 (8.59 - 8.78)	-0.097 ± 0.11 (-0.14 - -0.022)	0.393	-0.33, 0.14	(8.0 - 9.9)	(6.6-10.3)	(8.7-10.1)
Serine	4.90 ± 0.083 (4.83 - 4.97)	4.88 ± 0.083 (4.68 - 4.99)	0.017 ± 0.12 (-0.16 - 0.22)	0.888	-0.24, 0.27	(3.5 - 5.5)	(4.2-5.5)	(4.9-6.0)
Threonine	3.35 ± 0.040 (3.33 - 3.40)	3.38 ± 0.040 (3.31 - 3.50)	-0.029 ± 0.057 (-0.16 - 0.045)	0.616	-0.15, 0.095	(3.1 - 4.0)	(2.9-3.9)	(3.3-4.2)
Tryptophan	0.50 ± 0.012 (0.48 - 0.52)	0.51 ± 0.012 (0.49 - 0.53)	-0.0040 ± 0.012 (-0.032 - 0.030)	0.744	-0.030, 0.022	(0.4 - 0.8)	(0.5-1.2)	(0.4-1.0)
Tyrosine	3.17 ± 0.26 (2.36 - 3.73)	3.20 ± 0.26 (2.46 - 3.64)	-0.024 ± 0.36 (-0.51 - 0.42)	0.949	-0.82, 0.77	(2.1 - 4.0)	(2.9-4.7)	(3.7-4.3)
Valine	4.71 ± 0.046 (4.63 - 4.83)	4.69 ± 0.046 (4.62 - 4.76)	0.023 ± 0.065 (-0.060 - 0.10)	0.727	-0.12, 0.17	(3.9 - 5.5)	(2.1-5.2)	(4.2-5.3)

(continued over)

Table 6. Replicated Trial (Ohio): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Fatty acids (% of total)								
16:0 palmitic acid	8.94 ± 0.076 (8.67 - 9.11)	8.60 ± 0.076 (8.41 - 8.74)	0.34 ± 0.11 (-0.068 - 0.64)	0.007	0.11, 0.57	(8.8 - 13.8)	(7-19)	(9.9-12.0)
18:0 stearic acid	1.95 ± 0.018 (1.92 - 1.98)	1.85 ± 0.018 (1.79 - 1.91)	0.10 ± 0.025 (0.052 - 0.19)	0.001	0.049, 0.16	(1.4 - 2.6)	(1-3)	(1.4-2.2)
18:1 oleic acid	22.70 ± 0.22 (22.52 - 23.12)	22.79 ± 0.22 (22.15 - 23.40)	-0.083 ± 0.28 (-0.84 - 0.46)	0.774	-0.70, 0.53	(20.7 - 37.7)	(20-46)	(20.6-27.5)
18:2 linoleic acid	64.44 ± 0.27 (64.02 - 64.97)	64.75 ± 0.27 (64.16 - 65.65)	-0.31 ± 0.34 (-1.23 - 0.49)	0.380	-1.05, 0.43	(48.0 - 66.1)	(35-70)	(55.9-66.1)
18:3 linolenic acid	1.10 ± 0.020 (1.07 - 1.17)	1.12 ± 0.020 (1.07 - 1.20)	-0.015 ± 0.028 (-0.13 - 0.060)	0.606	-0.075, 0.046	(0.9 - 1.5)	(0.8-2)	(0.8-1.1)
20:0 arachidic acid	0.38 ± 0.0045 (0.37 - 0.39)	0.39 ± 0.0045 (0.38 - 0.39)	-0.0082 ± 0.0062 (-0.019 - 0.0057)	0.211	-0.022, 0.0054	(0.3 - 0.6)	(0.1-2)	(0.3-0.5)
20:1 eicosenoic acid	0.30 ± 0.0084 (0.29 - 0.32)	0.33 ± 0.0084 (0.31 - 0.34)	-0.026 ± 0.012 (-0.038 - 0.0061)	0.049	-0.052, -0.00012	(0.2 - 0.4)	(na)	(0.2-0.3)
22:0 behenic acid	0.18 ± 0.0043 (0.17 - 0.19)	0.18 ± 0.0043 (0.18 - 0.19)	-0.0016 ± 0.0061 (-0.0083 - 0.0078)	0.790	-0.015, 0.012	(0.1 - 0.3)	(na)	(0.1-0.3)

(continued over)

Table 6. Replicated Trial (Ohio): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Minerals								
Calcium (%)	0.0051 ± 0.00023 (0.0047 - 0.0056)	0.0049 ± 0.00023 (0.0046 - 0.0051)	0.00020 ± 0.00027 (-0.00021 -0.00057)	0.460	-0.00038, 0.00079	(0.003 - 0.009)	(0.01-0.1)	(0.003-0.006)
Copper (mg/kg dw)	1.48 ± 0.14 (1.19 - 1.83)	1.68 ± 0.14 (1.50 - 1.82)	-0.21 ± 0.19 (-0.63 - 0.23)	0.308	-0.63, 0.22	(0.9 - 2.8)	(0.9-10)	(na)
Iron (mg/kg dw)	19.99 ± 0.81 (19.08 - 21.27)	20.86 ± 0.81 (18.77 - 25.69)	-0.86 ± 1.14 (-4.42 - 0.54)	0.462	-3.35, 1.62	(11 - 49)	(1-100)	(na)
Magnesium (%)	0.13 ± 0.0027 (0.12 - 0.13)	0.13 ± 0.0027 (0.12 - 0.13)	0.0010 ± 0.0038 (-0.0098 - 0.010)	0.795	-0.0072, 0.0092	(0.08 - 0.2)	(0.09-1.0)	(na)
Manganese (mg/kg dw)	6.65 ± 0.29 (5.74 - 7.25)	6.07 ± 0.29 (5.66 - 6.90)	0.58 ± 0.40 (0.0057 - 1.34)	0.169	-0.29, 1.46	(2.6 - 7.8)	(0.7-54)	(na)
Phosphorus (%)	0.37 ± 0.0076 (0.34 - 0.39)	0.37 ± 0.0076 (0.35 - 0.38)	-0.0031 ± 0.011 (-0.040 - 0.025)	0.774	-0.027, 0.020	(0.24 - 0.43)	(0.26-0.75)	(0.31-0.36)
Potassium (%)	0.38 ± 0.0075 (0.37 - 0.39)	0.38 ± 0.0075 (0.36 - 0.41)	-0.0026 ± 0.011 (-0.014 - 0.022)	0.813	-0.026, 0.021	(0.29 - 0.53)	(0.32-0.72)	(na)
Zinc (mg/kg dw)	30.87 ± 1.02 (28.31 - 33.17)	30.92 ± 1.02 (29.53 - 33.26)	-0.048 ± 1.45 (-4.95 - 3.06)	0.973	-3.20, 3.10	(15 - 33)	(12-30)	(na)

(continued over)

Table 6. Replicated Trial (Ohio): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Fiber and Proximates								
Ash (% dw)	1.49 ± 0.069 (1.37 - 1.62)	1.56 ± 0.069 (1.41 - 1.75)	-0.067 ± 0.097 (-0.29 - 0.21)	0.503	-0.28, 0.14	(0.8 - 1.8)	(1.1-3.9)	(1.2-1.8)
Carbohydrates (% dw)	80.95 ± 0.19 (80.71 - 81.25)	81.11 ± 0.19 (80.23 - 81.52)	-0.15 ± 0.26 (-0.76 - 0.49)	0.560	-0.72, 0.41	(83.1 - 89.6)	(na)	(na)
ADF (% dw)	3.73 ± 0.26 (3.14 - 4.23)	3.86 ± 0.26 (3.07 - 4.28)	-0.13 ± 0.37 (-0.50 - 0.071)	0.727	-0.94, 0.68	(2.3 - 5.7)	(3.3 - 4.3)	(3.1 - 5.3)
NDF (% dw)	11.69 ± 1.50 (10.95 - 12.53)	12.04 ± 1.50 (8.68 - 15.42)	-0.35 ± 2.09 (-3.72 - 2.89)	0.868	-4.91, 4.21	(8.2 - 16.1)	(8.3-11.9)	(9.6 - 15.3)
Moisture (% fw)	9.86 ± 0.36 (9.22 - 11.10)	9.73 ± 0.36 (8.56 - 10.70)	0.13 ± 0.51 (-1.48 - 2.54)	0.805	-0.98, 1.23	(6.1 - 15.6)	(7-23)	(9.4 - 15.8)
Total fat (%)	3.30 ± 0.14 (2.92 - 3.79)	3.18 ± 0.14 (2.88 - 3.61)	0.12 ± 0.19 (-0.69 - 0.90)	0.547	-0.30, 0.54	(1.7 - 4.3)	(3.1-5.7, 2.9-6.1)	(2.4-4.2)
Protein (% dw)	14.25 ± 0.19 (13.95 - 14.77)	14.15 ± 0.19 (13.55 - 14.84)	0.10 ± 0.21 (-0.16 - 0.66)	0.640	-0.36, 0.57	(6.7 - 13.4)	(6.0 - 12.0, 9.7 - 16.1)	(9.0 - 13.6)

(continued over)

Table 6. Replicated Trial (Ohio): Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Miscellaneous								
Phytic Acid (%)	0.96 ± 0.041 (0.91 - 0.99)	0.86 ± 0.041 (0.81 - 0.95)	0.097 ± 0.058 (0.020 - 0.18)	0.122	-0.030, 0.22	(0.5 - 1.3)	(to 0.9%)	(na)
Trypsin Inhibitor (TIU/mg dw)	3.30 ± 0.31 (3.00 - 3.90)	3.58 ± 0.31 (3.08 - 4.24)	-0.28 ± 0.44 (-0.95 - 0.50)	0.541	-1.25, 0.69	(3.40 - 7.18)	(na)	(na)
Vitamin E (mg/g dw)	0.0092 ± 0.00034 (0.0084 - 0.010)	0.0098 ± 0.00034 (0.0091 - 0.011)	-0.00060 ± 0.00048 (-0.0024 - 0.00093)	0.231	-0.0016, 0.00044	(0.006 - 0.022)	(0.017- 0.047)	(0.008- 0.012)

[a]ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt; TIU = trypsin inhibitor units.
[b]The mean of four replicate values.
[c]S.E. = standard error of the mean.
[d]C.I. = confidence interval.
[e]Comm. = commercial. The range of sample values for commercial lines grown in 1998 (Sidhu *et al.*, 1999).
[f]Lit. = literature. For amino and fatty acids, Watson, 1982; for all other components, Watson, 1987; protein and fat second values from Jugenheimer, 1976.
[g]Rpt. = reported. For amino and fatty acids, range for five control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a,b; 1997a,b).
[h]For ash, moisture and total fat, range for five control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a,b; 1997a,b).
[i]For ADF and NDF, range for three control lines analysed in Monsanto trials conducted between 1994 and 1995 (Sanders *et al.*, 1996b; 1997a,b).
[j]For calcium and phosphorus, range for three control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a; 1997b).

Table 7. All Trials: Fiber and Proximate Content of Forage and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Commercial[e] (Range)	Reported[f] (Range)
Ash (% dw)	3.81 ± 0.46 (2.36 - 6.80)	4.02 ± 0.46 (2.46 - 6.28)	-0.21 ± 0.18 (-0.99 - 0.51)	0.249	-0.56, 0.15	(2.03 - 7.49)	(2.9 - 5.1)
Carbohydrates (% dw)	86.71 ± 0.76 (82.68 - 90.32)	87.11 ± 0.76 (83.71 - 90.03)	-0.40 ± 0.43 (-2.41 - 2.72)	0.363	-1.30, 0.50	(81.5 - 88.9)	(84.6 - 89.1)
ADF (% dw)	25.72 ± 1.30 (17.01 - 33.52)	24.84 ± 1.30 (19.53 - 31.83)	0.89 ± 0.88 (-4.15 - 8.05)	0.321	-0.89, 2.66	(17.6 - 34.5)	(21.4 - 29.2)
NDF (% dw)	42.09 ± 1.77 (36.39 - 49.03)	42.45 ± 1.77 (35.44 - 53.24)	-0.35 ± 1.21 (-4.21 - 10.56)	0.774	-2.87, 2.17	(29.6 - 50.7)	(39.9 - 46.6)
Moisture (% fw)	67.02 ± 1.91 (60.30 - 75.00)	66.24 ± 1.91 (61.00 - 73.70)	0.78 ± 0.58 (-2.30 - 5.20)	0.223	-0.63, 2.19	(47.0 - 78.8)	(68.7 - 73.5)
Protein (% dw)	7.14 ± 0.44 (5.57 - 8.98)	6.80 ± 0.44 (5.49 - 8.69)	0.34 ± 0.32 (-1.61 - 2.20)	0.292	-0.31, 0.99	(4.9 - 11.0)	(4.8 - 8.4)
Total fat (% dw)	2.36 ± 0.29 (0.69 - 3.64)	2.17 ± 0.29 (0.61 - 3.42)	0.20 ± 0.18 (-0.77 - 1.53)	0.299	-0.20, 0.59	(0.79 - 3.64)	(1.4 - 2.1)

[a]ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt.
[b]The mean of all values.
[c]S.E. = standard error of the mean.
[d]C.I. = confidence interval.
[e]The range of sample values across commercial lines grown in 1998 (Sidhu *et al.*, 1999).
[f]Range for two control lines analyzed in Monsanto Company trials conducted in 1994 and 1995 (Sanders *et al.*, 1996b; 1997a).

Amendment 1

Table 8. All Trials: Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Amino acids (% of total)								
Alanine	7.93 ± 0.064 (7.78 - 8.22)	7.89 ± 0.064 (7.65 - 8.17)	0.036 ± 0.036 (-0.13 - 0.28)	0.351	-0.046, 0.12	(7.1 - 8.2)	(6.4-9.9)	(7.3-8.8)
Arginine	4.16 ± 0.10 (3.79 - 4.49)	4.24 ± 0.10 (3.90 - 4.63)	-0.076 ± 0.081 (-0.46 - 0.27)	0.371	-0.25, 0.10	(4.0 - 5.5)	(2.9-5.9)	(3.6-5.0)
Aspartic acid	6.45 ± 0.035 (6.29 - 6.62)	6.40 ± 0.035 (6.18 - 6.56)	0.057 ± 0.040 (-0.17 - 0.19)	0.159	-0.023, 0.14	(6.3 - 7.4)	(5.8-7.2)	(6.3-7.5)
Cystine	2.00 ± 0.065 (1.69 - 2.27)	2.00 ± 0.065 (1.63 - 2.22)	0.0037 ± 0.058 (-0.38 - 0.52)	0.948	-0.12, 0.12	(1.8 - 2.9)	(1.2-1.6)	(1.8-2.7)
Glutamic acid	19.84 ± 0.16 (19.16 - 20.47)	19.81 ± 0.16 (19.19 - 20.41)	0.037 ± 0.12 (-0.44 - 0.54)	0.768	-0.22, 0.30	(17.4 - 20.1)	(12.4-19.6)	(19.5-22.8)
Glycine	3.49 ± 0.073 (3.22 - 3.74)	3.51 ± 0.073 (3.22 - 3.86)	-0.024 ± 0.056 (-0.35 - 0.24)	0.682	-0.15, 0.10	(3.4 - 4.6)	(2.6-4.7)	(3.2-4.2)
Histidine	2.72 ± 0.043 (2.45 - 2.81)	2.74 ± 0.043 (2.56 - 2.88)	-0.018 ± 0.024 (-0.13 - 0.10)	0.477	-0.071, 0.036	(2.6 - 3.4)	(2.0-2.8)	(2.8-3.3)
Isoleucine	3.87 ± 0.037 (3.59 - 4.06)	3.80 ± 0.037 (3.65 - 3.93)	0.065 ± 0.034 (-0.060 - 0.19)	0.071	-0.0063, 0.14	(3.0 - 4.1)	(2.6-4.0)	(3.2-4.3)
Leucine	14.20 ± 0.19 (13.63 - 14.79)	14.07 ± 0.19 (13.59 - 14.60)	0.12 ± 0.14 (-0.52 - 0.99)	0.399	-0.18, 0.42	(11.3 - 14.4)	(7.8-15.2)	(12.6-15.8)

(continued over)

Amendment 1

Table 8. All Trials: Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Lysine	2.69 ± 0.078 (2.42 - 2.96)	2.67 ± 0.078 (2.35 - 3.00)	0.024 ± 0.066 (-0.36 - 0.30)	0.727	-0.12, 0.17	(2.6 - 3.9)	(2.0-3.8)	(2.6-3.5)
Methionine	1.94 ± 0.053 (1.76 - 2.16)	2.03 ± 0.053 (1.74 - 2.21)	-0.097 ± 0.061 (-0.41 - 0.42)	0.125	-0.22, 0.029	(1.6 - 2.9)	(1.0-2.1)	(1.3-2.6)
Phenylalanine	5.32 ± 0.047 (5.18 - 5.52)	5.24 ± 0.047 (5.09 - 5.36)	0.075 ± 0.035 (-0.10 - 0.21)	0.052	-0.00082, 0.15	(4.7 - 5.5)	(2.9-5.7)	(5.0-6.1)
Proline	8.88 ± 0.078 (8.44 - 9.10)	8.96 ± 0.078 (8.59 - 9.26)	-0.076 ± 0.049 (-0.35 - 0.25)	0.129	-0.17, 0.023	(8.0 - 9.9)	(6.6-10.3)	(8.7-10.1)
Serine	4.87 ± 0.043 (4.72 - 5.09)	4.86 ± 0.043 (4.68 - 4.99)	0.010 ± 0.049 (-0.18 - 0.25)	0.839	-0.091, 0.11	(3.5 - 5.5)	(4.2-5.5)	(4.9-6.0)
Threonine	3.37 ± 0.026 (3.26 - 3.46)	3.33 ± 0.026 (3.19 - 3.50)	0.036 ± 0.030 (-0.16 - 0.14)	0.246	-0.026, 0.098	(3.1 - 4.0)	(2.9-3.9)	(3.3-4.2)
Tryptophan	0.53 ± 0.013 (0.44 - 0.58)	0.54 ± 0.013 (0.48 - 0.60)	-0.015 ± 0.014 (-0.11 - 0.072)	0.274	-0.044, 0.014	(0.4 - 0.8)	(0.5-1.2)	(0.4-1.0)
Tyrosine	3.02 ± 0.14 (2.36 - 3.73)	3.25 ± 0.14 (2.43 - 3.64)	-0.23 ± 0.17 (-1.12 - 0.42)	0.195	-0.58, 0.12	(2.1 - 4.0)	(2.9-4.7)	(3.7-4.3)
Valine	4.74 ± 0.032 (4.59 - 4.85)	4.71 ± 0.032 (4.62 - 4.94)	0.031 ± 0.040 (-0.094 - 0.16)	0.450	-0.052, 0.11	(3.9 - 5.5)	(2.1-5.2)	(4.2-5.3)

(continued over)

Table 8. All Trials: Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Fatty acids (% of total)								
16:0 palmitic acid	9.13 ± 0.083 (8.67 - 9.57)	8.89 ± 0.083 (8.41 - 9.44)	0.24 ± 0.054 (-0.068 - 0.64)	<0.001	0.12, 0.35	(8.8 - 13.8)	(7-19)	(9.9-12.0)
18:0 stearic acid	1.92 ± 0.039 (1.80 - 2.06)	1.83 ± 0.039 (1.67 - 1.98)	0.094 ± 0.025 (-0.066 - 0.19)	0.001	0.041, 0.15	(1.4 - 2.6)	(1-3)	(1.4-2.2)
18:1 oleic acid	22.40 ± 0.24 (21.37 - 23.12)	23.08 ± 0.24 (22.15 - 24.14)	-0.68 ± 0.23 (-2.27 - 0.46)	0.007	-1.15, -0.20	(20.7 - 37.7)	(20-46)	(20.6-27.5)
18:2 linoleic acid	64.62 ± 0.28 (63.79 - 65.80)	64.26 ± 0.28 (63.07 - 65.65)	0.35 ± 0.25 (-1.23 - 2.23)	0.172	-0.17, 0.87	(48.0 - 66.1)	(35-70)	(55.9-66.1)
18:3 linolenic acid	1.11 ± 0.011 (1.07 - 1.17)	1.11 ± 0.011 (1.07 - 1.20)	0.00027 ± 0.014 (-0.13 - 0.060)	0.985	-0.031, 0.032	(0.9 - 1.5)	(0.8-2)	(0.8-1.1)
20:0 arachidic acid	0.36 ± 0.0083 (0.34 - 0.39)	0.37 ± 0.0083 (0.33 - 0.40)	-0.0029 ± 0.0041 (-0.019 - 0.016)	0.489	-0.012, 0.0058	(0.3 - 0.6)	(0.1-2)	(0.3-0.5)
20:1 eicosenoic acid	0.29 ± 0.0072 (0.28 - 0.32)	0.30 ± 0.0072 (0.27 - 0.34)	-0.013 ± 0.0069 (-0.038 - 0.019)	0.066	-0.028, 0.00098	(0.2 - 0.4)	(na)	(0.2-0.3)
22:0 behenic acid	0.16 ± 0.0048 (0.14 - 0.19)	0.16 ± 0.0048 (0.14 - 0.19)	-0.0019 ± 0.0033 (-0.010 - 0.011)	0.564	-0.0085, 0.0047	(0.08 - 0.3)	(na)	(0.1-0.3)

(continued over)

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Table 8. All Trials: Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Minerals								
Calcium (%)	0.0047 ± 0.00026 (0.0037 - 0.0056)	0.0046 ± 0.00026 (0.0033 - 0.0058)	0.00017 ± 0.00016 (-0.00050 -0.00091)	0.286	-0.00016, 0.00050	(0.003 - 0.009)	(0.01-0.1)	(0.003-0.006)
Copper (mg/kg dw)	1.79 ± 0.11 (1.19 - 2.37)	1.90 ± 0.11 (1.50 - 2.33)	-0.11 ± 0.13 (-0.63 - 0.36)	0.399	-0.38, 0.16	(0.9 - 2.8)	(0.9-10)	(na)
Iron (mg/kg dw)	22.71 ± 0.88 (19.08 - 25.94)	22.95 ± 0.88 (18.77 - 26.62)	-0.24 ± 0.49 (-4.42 - 2.18)	0.627	-1.21, 0.74	(11 - 49)	(1-100)	(na)
Magnesium (%)	0.12 ± 0.0023 (0.11 - 0.13)	0.12 ± 0.0023 (0.11 - 0.13)	0.00028 ± 0.0022 (-0.016 - 0.010)	0.901	-0.0046, 0.0052	(0.08 - 0.2)	(0.09-1.0)	(na)
Manganese (mg/kg dw)	6.47 ± 0.54 (4.64 - 9.63)	6.55 ± 0.54 (4.96 - 8.83)	-0.081 ± 0.27 (-0.88 - 1.34)	0.768	-0.65, 0.48	(2.6 - 7.8)	(0.7-54)	(na)
Phosphorus (%)	0.36 ± 0.0053 (0.32 - 0.39)	0.36 ± 0.0053 (0.32 - 0.39)	-0.0033 ± 0.0059 (-0.042 - 0.025)	0.584	-0.016, 0.0093	(0.24 - 0.43)	(0.26-0.75)	(0.31-0.36)
Potassium (%)	0.36 ± 0.0068 (0.35 - 0.39)	0.36 ± 0.0068 (0.34 - 0.41)	-0.0018 ± 0.0068 (-0.039 - 0.022)	0.791	-0.016, 0.012	(0.29 - 0.53)	(0.32-0.72)	(na)
Zinc (mg/kg dw)	28.35 ± 1.42 (20.23 - 33.17)	28.72 ± 1.42 (23.47 - 33.26)	-0.37 ± 0.64 (-4.95 - 4.14)	0.566	-1.66, 0.92	(15 - 33)	(12-30)	(na)

(continued over)

Table 8. All Trials: Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Fiber and Proximates								
Ash (% dw)	1.45 ± 0.035 (1.28 - 1.62)	1.49 ± 0.035 (1.32 - 1.75)	-0.044 ± 0.043 (-0.29 - 0.21)	0.326	-0.14, 0.048	(0.8 - 1.8)	(1.1-3.9)	(1.2-1.8)
Carbohydrates (% dw)	82.76 ± 0.51 (80.71 - 84.33)	82.29 ± 0.51 (80.23 - 83.70)	0.47 ± 0.29 (-1.60 - 2.01)	0.117	-0.13, 1.08	(83.1 - 89.6)	(na)	(na)
ADF (% dw)	3.72 ± 0.22 (3.14 - 5.17)	3.60 ± 0.22 (2.79 - 4.28)	0.12 ± 0.20 (-0.71 - 1.48)	0.578	-0.32, 0.55	(2.3 - 5.7)	(3.3 - 4.3)	(3.1 - 5.3)
NDF (% dw)	10.06 ± 0.74 (7.89 - 12.53)	10.00 ± 0.74 (8.25 - 15.42)	0.057 ± 0.76 (-3.72 - 2.89)	0.940	-1.47, 1.59	(8.2 - 16.1)	(8.3-11.9)	(9.6 - 15.3)
Moisture (% fw)	11.13 ± 0.51 (9.01 - 13.30)	11.78 ± 0.51 (8.56 - 14.80)	-0.66 ± 0.35 (-2.60 - 2.54)	0.079	-1.40, 0.088	(6.1 - 15.6)	(7-23)	(9.4 - 15.8)
Total fat (%)	3.61 ± 0.12 (2.92 - 3.94)	3.67 ± 0.12 (2.88 - 4.13)	-0.058 ± 0.091 (-0.69 - 0.90)	0.524	-0.24, 0.12	(1.7 - 4.3)	(3.1-5.7, 2.9-6.1)	(2.4-4.2)
Protein (% dw)	12.20 ± 0.59 (10.30 - 14.77)	12.60 ± 0.59 (11.02 - 14.84)	-0.40 ± 0.30 (-1.62 - 1.42)	0.192	-1.03, 0.22	(6.7 - 13.4)	(6.0 - 12.0, 9.7 - 16.1)	(9.0 - 13.6)

(continued over)

Table 8. All Trials: Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Phytic Acid, Trypsin Inhibitor and Vitamin E Content of Grain and Statistical Summary

Component[a]	NK603 Mean[b] ± S.E.[c] (Range)	Control Mean[b] ± S.E.[c] (Range)	Difference (NK603 minus Control) Mean ± S.E.[c] (Range)	p-value	95% C.I.[d] (Lower, Upper)	Comm.[e] (Range)	Lit.[f] (Range)	Rpt.[g,h,i,j] (Range)
Miscellaneous								
Phytic Acid (%)	0.97 ± 0.032 (0.70 - 1.06)	1.00 ± 0.032 (0.81 - 1.21)	-0.029 ± 0.040 (-0.29 - 0.18)	0.481	-0.12, 0.059	(0.5 - 1.3)	(to 0.9%)	(na)
Trypsin Inhibitor (TIU/mg dw)	3.16 ± 0.30 (2.34 - 5.08)	2.67 ± 0.30 (1.39 - 5.14)	0.49 ± 0.34 (-2.15 - 2.84)	0.149	-0.18, 1.17	(3.40 - 7.18)	(na)	(na)
Vitamin E (mg/g dw)	0.0088 ± 0.00039 (0.0070 - 0.010)	0.0090 ± 0.00039 (0.0064 - 0.011)	-0.00015 ± 0.00028 (-0.0024 - 0.0013)	0.602	-0.00075, 0.00046	(0.006 - 0.022)	(0.017- 0.047)	(0.008- 0.012)

[a]ADF = acid detergent fiber; NDF = neutral detergent fiber; dw = dry wt.; fw = fresh wt; TIU = trypsin inhibitor units.
[b]The mean of all values.
[c]S.E. = standard error of the mean.
[d]C.I. = confidence interval.
[e]Comm. = commercial. The range of sample values for commercial lines grown in 1998 (Sidhu *et al.*, 1999).
[f]Lit. = literature. For amino and fatty acids, Watson, 1982; for all other components, Watson, 1987; protein and fat second values from Jugenheimer, 1976.
[g]Rpt. = reported. For amino and fatty acids, range for five control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a,b; 1997a,b).
[h]For ash, moisture and total fat, range for five control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a,b; 1997a,b).
[i]For ADF and NDF, range for three control lines analysed in Monsanto trials conducted between 1994 and 1995 (Sanders *et al.*, 1996b; 1997a,b).
[j]For calcium and phosphorus, range for three control lines analysed in Monsanto trials conducted between 1993 and 1995 (Sanders and Patzer, 1995; Sanders *et al.*, 1996a; 1997b).

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