Application for renewal of authorization of Bt11 maize and derived products notified according to Articles 11 and 23 of Regulation (EC) No 1829/2003 on genetically modified food and feed

PART I: TECHNICAL DOSSIER

APPENDIX 8

Feeding studies

Application for renewal of authorization of Bt11 maize and derived products notified according to Articles 11 and 23 of Regulation (EC) No 1829/2003 on genetically modified food and feed

PART I: TECHNICAL DOSSIER

APPENDIX 8.1

An evaluation of laying hens (Gallus gallus) fed diets containing transgenic event Bt176 and Bt11 maize (corn)

AN EVALUATION OF LAYING HENS (*Gallus gallus*) FED DIETS CONTAINING TRANSGENIC EVENT 176 AND Bt11 MAIZE (CORN)

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 108-394

AUTHORS: [redacted]

STUDY INITIATION: August 12, 1997

STUDY COMPLETION: December 12, 1997

<u>SPONSOR</u>

Novartis Seeds
3054 Cornwallis Road
Research Triangle Park, North Carolina 27709

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

REPORT APPROVAL

SPONSOR: Novartis Seeds
3054 Cornwallis Road
Research Triangle Park, North Carolina 27709

TITLE: An Evaluation of Laying Hens (*Gallus gallus*) Fed Diets Containing Transgenic Event 176 and Bt11 Maize (Corn)

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 108-394

STUDY DIRECTOR:

[redacted]

12-12-97
Date

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12/12/97
Date

SPONSOR'S REPRESENTATIVE:

[redacted]

12/15/97
Date

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SUMMARY

SPONSOR: Novartis Seeds

TEST SUBSTANCE: Transgenic Corn - Event 176-Derived Hybrid
Transgenic Corn - Event Bt11-Derived Hybrid

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 108-394

STUDY: An Evaluation of Laying Hens (*Gallus gallus*) Fed Diets Containing Transgenic Event 176 and Bt11 Maize (Corn)

RESULTS: Laying hens fed diets containing 64% corn meal in the diet from Event 176-derived or Event Bt11-derived transgenic corn showed no effect upon survivability, health, egg production or egg weight when compared to birds fed diets containing the non- transgenic control corn meal. There were no differences from the pretreatment phase in the parameters measured. Additionally, the CryIA(b) and PAT transgenic proteins were not detected in any of the five tissue types (egg white, egg yolk, liver, breast and thigh) analyzed.

TEST DATES: Hatch - January 24, 1997
Acclimation - July 25, 1997
Pre- treatment Phase - August 5, 1997
Experimental Start - August 12, 1997
Termination In-Life Phase - August 26, 1997
Experimental Termination - December 8,1997

NOMINAL TEST
CONCENTRATION: 64 % (w/w) of the diet

TEST ANIMALS: Single comb, white laying hens (*Gallus gallus*)

AGE TEST ANIMALS: 28 weeks of age at test initiation.

SOURCE TEST ANIMALS: Truslow Farms, Incorporated
510 Truslow Road
Chestertown, Maryland 21620

STUDY COMPLETION: December 12, 1997

INTRODUCTION

This study was conducted by Wildlife International Ltd. for Novartis Seeds at the Wildlife International Ltd. toxicology facility in Easton, Maryland. The in-life portion of the test was conducted from August 12, 1997, to August 26, 1997. Raw data generated at Wildlife International Ltd. and a copy of the final report are filed under Project Number 108-394 in archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of this study was to evaluate general health and egg production parameters in laying hens fed diets prepared with transgenic maize (corn) for 14 days, and to evaluate selected tissues and eggs for the presence of the transgenic proteins CryIA(b) and PAT[1]

MATERIALS AND METHODS

The methods, species used and route of administration described in this protocol are based in part upon procedures specified in Section 171-4 of the Environmental Protection Agency's Registration Guidelines, *Pesticide Assessment Guidelines, FIFRA Subdivision O, Hazard Evaluation: Pesticide-Residue Chemistry Guidelines* (1) and modified to suit the objectives of the study.

Single comb, white laying hens were administered 64% transgenic corn meal as a portion of their diet. One group received a diet prepared with grain from Event 176-derived hybrid corn plants (MAX 454**) ,** and a second group received a diet prepared with grain from Bt11- derived hybrid corn plants (N4640Bt). A third group received a diet prepared with grain from the non-transgenic control hybrid (Event 176) corresponding to the Event 176-derived hybrid (G4494)**,** and a fourth group received a diet prepared with grain from the non- transgenic control hybrid (Event Bt11) from the Bt11-derived hybrid (N4640). The birds were fed the diets *ad libitum* for 14 days, and evaluated for survival, body weight, and general health. The number and weight of eggs produced were measured daily. Data on feed consumption, egg production and egg weight for each pen was compared to the comparable values from the pre-treatment phase (final 7 days of acclimation). Eggs from the final two days of the study were collected for analysis of the transgenic proteins. Hens from the control and treatment groups were sacrificed at the end of the 14 day exposure period and selected tissues were taken for analysis of the transgenic proteins.

Test Substance

[1] Transgenic maize hybrids derived from the transformation events designated as "176" and "Bt11" have been genetically modified to produce CryIA(b) insecticidal protein and PAT (phosphinothricin acetyltransferase) protein.

The control and test substances were received from Novartis Seeds. Whole corn seed identified as Isogenic Control Corn (Non-Transgenic Control Corn for Event Bt11 Hybrid) and Bt11 Corn Grain (Transgenic Corn - Event Bt11-Derived Hybrid) were received on July 16, 1997, and assigned Wildlife International Ltd. Identification numbers 4147 and 4148, respectively. Whole seed corn identified as Bt 176 Grain (Transgenic Corn - Event 176-Derived Hybrid) and Control Grain (Non-Transgenic Control Corn for Event 176 Hybrid) were received on July 21, 1997, and assigned Wildlife International Ltd. Identification numbers 4153 and 4154, respectively. The grain was taken to Parc's Feed Mill, Harmony, MD and ground to produce a fine corn meal using a Star Trac roller mill. Before the first lot was ground and between each different type of corn seed, the roller mill was prepared by processing a batch of commercial corn seed through the mill, which was discarded. The mill was then disassembled, cleaned and reassembled prior to grinding the next lot of corn.

Test Birds and Treatment Groups

Single comb, white laying hens (*Gallus gallus*) were obtained from Truslow Farm, Chestertown, Maryland. Hens were 28 weeks of age at the initiation of treatment and weighed between 1124 and 1716 grams. All study birds were from the same lot and age and were acclimated to the test facility for 11 days prior to the start of the seven day pre-treatment phase. Any birds exhibiting signs of debilitating physical injury or abnormal behavior were rejected for use. Of the population eligible for study use, the 40 hens with the highest or most consistent egg production were selected for use in the study. These birds were randomly assigned to the control and treatment groups. Each bird was identified by a uniquely numbered leg band. Each pen was identified by a unique pen number, project number and test concentration. Birds were grouped as follows:

Group	Treatment Group	No. of Hens
1	Non-Transgenic Control Corn for Event 176 Hybrid	10
2	Transgenic Corn - Event 176-Derived Hybrid	10
3	Non-Transgenic Control Corn for Event Bt11 Hybrid	10
4	Transgenic Corn - Event Bt11-Derived Hybrid	10

Each group was fed the appropriate test or control diet *ad libitum* for 14 days. Control birds received an amount of the non-transgenic corn equivalent to the amount in the transgenic diets.

Duration of the Study

The primary phases of this study and their durations were:

1. Acclimation - 18 days.
   a. Stabilization - 11 days
   b. Pre-treatment - 7 days.
2. Administration of treated diet and egg collection - 14 days.
3. Collection of tissue samples - at the end of the 14 day exposure period.

Animal Diet

Hens were fed a commercial non-medicated laying mash that contained 64% corn meal (Appendix I) during both the acclimation and pre-treatment phases. A ration of this type, using 64% corn, is typical of that used for laying hens in the United States, and is at or above the proportion of corn typically used in laying rations in Europe (2). During the treatment phase of the study, the non-transgenic (control) or transgenic (treatment) corn meal replaced the corn component of the diet. Water and feed were available *ad libitum* during acclimation, pre-treatment and testing. The birds were not given antibiotics or other therapeutic agents in the diet during the study.

Diet Preparation

The diets were prepared at Wildlife International Ltd. by mixing the transgenic or non-transgenic corn meal directly into the feed with a Patterson Kelly twinshell bulk blender. Fresh diets were prepared weekly (Appendix II).

Housing and Environmental Conditions

The adult birds were housed in battery breeding pens manufactured by Safeguard Products, Inc. (Model No. 5355) or equivalent. Each pen had floor space that measured approximately 75 X 90 cm. Ceiling height measured approximately 45 cm. External walls, ceilings and floors of each breeder pen were constructed of vinyl-coated wire grid. Each pen housed one hen. During the pre-treatment phase the average ambient room temperature was 23.5 ± 0.6 C (SD) with an average relative humidity of 66% ± 14% (SD). During the exposure phase of the test the average ambient room temperature was 23.4 ± 0.8 C (SD) with an average relative humidity of 71% ± 15% (SD).

During acclimation and the first day of pre-treatment the photoperiod was 17 hours of light per day. During the remainder of the pre-treatment phase and throughout the test the photoperiod was 18 hours of light per day. Lighting was provided by fluorescent lights which closely approximate noon-day sunlight (noon-day sun - 4870°K, Chroma 50 or equivalent - 5000°K). The birds were exposed to an average of approximately 189 ± 72 lux of illumination. Housing and husbandry practices were conducted so as to adhere to the guidelines established by the National Research Council (3).

Observations

During acclimation and testing all birds were observed daily for mortality and general health. During the study any clinical observations, including overt signs of toxicity, were recorded.

Body Weights and Feed Consumption

Individual body weight measurements were taken at test initiation, on Day 7 of Week 1 and at test termination on Day 7 of Week 2. Feed consumption for each pen was measured daily during the seven day pre-treatment period, and throughout the test.

Egg Production and Egg Weights

The number of eggs laid per hen was recorded daily during both the seven day pre-treatment period and the test period. Additionally, the weight of each egg laid during those two periods was recorded.

Analysis of Samples

The methodology used to analyze the test samples and the results of these analyses were documented by the Sponsor (Appendix VII).

Diet Samples - Following milling, a fifty gram sample was collected from Transgenic Corn - Event 176-Derived Hybrid, Transgenic Corn - Event Bt11-Derived Hybrid, and their corresponding non-transgenic controls. Samples were also collected from each batch of final prepared diet. These samples were shipped to the Sponsor to assess concentrations of transgenic proteins. All samples were collected into appropriate labeled sample containers and stored frozen (7.6-12.7°F) until shipped to the sponsor on dry ice.

Egg Samples - For each hen, condition of egg, egg production and the weight of each egg produced was recorded daily during the seven day pre-treatment period and during the study. Beginning one day prior to test termination, eggs were collected twice a day. Eggs were opened and egg yolks and whites were collected separately by pen and placed into appropriately labeled polyethylene bottles. Shells were discarded. Samples were stored frozen (8.1-16.4°F) prior to shipment on dry ice to the Sponsor.

Tissue Samples - At the end of the 14-day exposure period, all birds from each treatment and each control group were euthanized using carbon dioxide gas. Samples of liver, breast and thigh tissues were collected. Tissue samples from each hen were maintained separately, and placed in appropriately labeled containers. Samples were stored frozen (8.1-17.0°F) prior to shipment on dry ice to the Sponsor.

Statistical Analysis

Upon completion of the test, an analysis of variance (ANOVA), followed by Tukey's Multiple Comparison procedure were used to determine if the differences observed between groups were statistically significant. Comparisons for this study were performed as two-tailed tests with $\alpha = 0.05$. Sample units were the individual pens within each experimental group. The group mean body weights were examined at each weighing interval, and change in body weight over the exposure period also was examined. Feed consumption was evaluated for any statistical differences between groups during the exposure, as well as the differences between pre- and post- treatment periods.

Egg production and egg weight parameters were evaluated for any statistical differences between groups during the exposure period as well as differences between pre- and post- treatment periods. The "delta", or change in parameter was determined for each pen for both the number of eggs laid per day, and average egg weight. The groups were then compared using an analysis of variance, and Tukey's Multiple comparison procedure to determine if statistically significant differences existed (4,5).

RESULTS

Mortalities and Clinical Observations

There were no mortalities observed in the control groups or in the treatment groups during the course of the study. Incidental clinical observations normally associated with handling and/or penwear such as lameness were observed in all groups. No clinical signs of toxicity were observed. Except for incidental observations, all birds appeared normal throughout the study.

Body Weight and Feed Consumption

There were no treatment related effects upon body weight or feed consumption in any of the treatment groups tested. There were no statistically significant differences in body weight or feed consumption between the groups fed the non-transgenic control corn and the respective transgenic maize. A slight reduction in body weight and feed consumption was observed in a number of birds from all groups during the treatment phase. This slight reduction was attributed to the stress of handling and is not treatment related (Tables 1 and 2, Appendices III and IV).

Egg Production and Egg Weight

There were no treatment related effects upon either egg production or egg weight in any of the treatment groups tested. There were no statistically significant differences in egg production or egg weight between the groups fed the non-transgenic control corn and the respective transgenic counterparts. Two hens in the Non-Transgenic Control for Event Bt11 Hybrid group produced soft shelled eggs. Of these, one bird began laying soft shelled eggs on the last day of the pre-treatment phase, and the other began following the Day 7 body weight measurement. In both instances, stress of handling may have contributed to the production of soft shelled eggs (Tables 3 and 4, Appendices V and VI).

Analysis of Samples

The results of the test sample analyses were documented by the sponsor and are presented in Appendix VII.

CONCLUSION

Laying hens fed diets containing 64% corn meal in the diet from Event 176-derived or Event Bt11-derived transgenic corn showed no effect upon survivability, health, egg production or egg weight when compared to birds fed diets containing the non- transgenic control corn meal. There were no differences from the pretreatment phase in the parameters measured. Additionally, the CryIA(b) and PAT transgenic proteins were not detected in any of the five tissue types (egg white, egg yolk, liver, breast and thigh) analyzed.

REFERENCES

1 **U.S. Environmental Protection Agency.** 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision O, Hazard Evaluation: Pesticide-Residue Chemistry Guidelines*, subsection 171-4, Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C.

2 **Personal Communication.** 21 & 22 July, 1997. Robert Stock, Ph.D., Poultry Nutritionist. Agway, Inc., Ithaca, NY.

3 **National Research Council.** 1996. *Guide for the Care and Use of Laboratory Animals.* Washington, D.C.: National Academy Press. 125 pp.

4 **Zar, Jerrold H.** 1974. *Biostatistical Analysis.* Prentice-Hall, Inc. Englewood Hills, NJ.

5 **Kirk, Roger E.** 1982. *Experimental Design: Procedures for the Behavioral Sciences*. Brooks/Cole Publishing Company. Monterey, CA.

TABLE 1
Mean Body Weight (g) from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt11 Maize (Corn)

	Day 0	Change Day 0-Day 7	Day 7	Change Day 7-Day 14	Day 14	Total Change
Non-transgenic Control Corn for Event 176 Hybrid						
Mean	1451.1	-8	1443.1	-80.1	1363	-88.1
SD	89.1433926	30.7209664	90.5826204	74.4975018	94.3103623	87.8160325
Transgenic Corn - Event 176-Derived Hybrid						
Mean	1395	-26.6	1368.4	-9	1359.4	-35.6
SD	140.669036	102.669048	100.05132	65.4047229	93.0247279	59.9707336
Non-transgenic Control Corn for Event Bt11 Hybrid						
Mean	1380.2	25.9	1406.1	-39.1	1367	-13.2
SD	152.762561	83.8827886	125.542777	37.0658573	125.797015	81.0744514
Transgenic Corn - Event Bt11-Derived Hybrid						
Mean	1426.6	-13.9	1412.7	-22.2	1390.5	-36.1
SD	106.210692	61.9129317	117.365573	30.5970224	121.578735	66.5306112

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

TABLE 2a
Mean Feed Consumption (g/bird/day) from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn) (Pre-Treatment)

Pre-Treatment

	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Mean
Non-transgenic Control Corn for Event 176 Hybrid								
Mean	145	137.7	115.4	136.8	97.8	99.5	89.8	117.42857
SD	19	30.346334	20.326228	23.13151	15.4905	13.16772	15.8871	10.421285
Transgenic Corn - Event 176-Derived Hybrid								
Mean	141	131	110.3	121	94.2	98.5	78	110.51429
SD	23	17.549929	18.366939	11.74734	16.1576	10.66927	16.95091	9.7739067
Non-transgenic Control Corn for Event Bt11 Hybrid								
Mean	138	145.1	107.7	128.3	96.6	94.3	77.3	112.4
SD	15	31.680523	15.599501	21.52544	12.1124	15.40599	29.113	14.798541
Transgenic Corn - Event Bt11-Derived Hybrid								
Mean	139	147.9	117.6	116.6	91.1	107.7	90	115.68571
SD	27	17.220466	12.112436	13.3766	19.4048	21.21347	12.5344	13.282693

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

TABLE 2b
Mean Feed Consumption (g/bird/day) from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn) (Exposure)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Mean
Non-transgenic Control Corn for Event 176 Hybrid															
Mean	86.8	85	83	104	108	95.6	107	88.6	97.1	99.7	92	87	83.3	91.8	93.5
SD	36.8	18	22	23	12.9	18.2	13.4	30.5	22	29.7	41	31.7	30.5	25.2	15.5
Transgenic Corn - Event 176-Derived Hybrid															
Mean	67.6	73	84	102	98.4	92.6	107	82.9	97.9	110	112	116	99.7	108	96.6
SD	35.5	31	29	26	24.7	16.9	24.7	27.2	28.9	22.5	18	23	8.5	22.3	14.7
Non-transgenic Control Corn for Event Bt11 Hybrid															
Mean	69	75	79	106	93.7	90.9	128	80	101	110	107	106	95	101	95.8
SD	39.3	15	14	26	15	17.6	35	18.8	17.9	30.8	7.8	13.7	14.6	13.4	12.1
Transgenic Corn - Event Bt11-Derived Hybrid															
Mean	80.8	90	89	119	85.6	99.3	118	81.3	102	112	113	106	99.9	102	99.7
SD	28.9	15	22	26	17.4	13.4	15.4	32.2	19.8	16.8	8.7	8.93	9.21	9.68	8.58

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

TABLE 3a

Mean Egg Production (Egg/Hen/Day) from an Evaluation of Laying Hens (*Gallus gallus*) Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn) (Pre-Treatment)

	Pre-Treatment							
	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Mean
Non-Transgenic Control Corn for Event 176 Hybrid								
Mean	0.40	0.80	0.90	0.80	0.90	1.00	1.00	0.83
SD	0.52	0.42	0.32	0.42	0.32	0.00	0.47	0.18
Transgenic Corn - Event 176-Derived Hybrid								
Mean	0.70	0.60	1.00	0.80	0.90	0.80	1.00	0.83
SD	0.48	0.52	0.47	0.42	0.57	0.42	0.00	0.18
Non-Transgenic Control Corn for Event Bt11 Hybrid								
Mean	0.90	0.60	0.80	0.70	0.70	0.70	0.90	0.76
SD	0.32	0.52	0.42	0.48	0.48	0.48	0.57	0.21
Transgenic Corn - Event Bt11-Derived Hybrid								
Mean	0.70	0.90	0.80	0.90	0.70	0.70	1.00	0.81
SD	0.48	0.57	0.42	0.32	0.48	0.48	0.00	0.22

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

TABLE 3b
Mean Egg Production (Egg/Hen/Day) from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn) (Exposure)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Mean
Non-Transgenic Control Corn for Event 176 Hybrid															
Mean	0.60	1.00	1.00	1.00	0.90	1.00	0.70	0.70	0.80	1.00	0.90	0.70	0.80	0.90	0.86
SD	0.52	0.00	0.00	0.47	0.32	0.00	0.48	0.48	0.42	0.00	0.32	0.67	0.42	0.74	0.16
Transgenic Corn - Event 176-Derived Hybrid															
Mean	0.90	0.80	1.00	0.80	0.70	0.90	0.90	0.60	0.70	0.90	0.70	0.90	0.60	1.20	0.83
SD	0.57	0.63	0.47	0.42	0.48	0.32	0.32	0.52	0.48	0.32	0.48	0.32	0.52	0.63	0.26
Non-Transgenic Control Corn for Event Bt11 Hybrid															
Mean	0.60	0.80	0.70	0.80	0.90	0.60	0.90	0.80	0.70	0.70	0.80	0.80	0.90	1.00	0.79
SD	0.52	0.42	0.48	0.42	0.57	0.52	0.57	0.42	0.48	0.48	0.42	0.42	0.32	0.47	0.32
Transgenic Corn - Event Bt11-Derived Hybrid															
Mean	1.10	0.80	1.00	1.00	1.00	0.70	0.90	0.90	1.00	1.00	0.80	1.00	0.70	1.00	0.92
SD	0.32	0.42	0.00	0.00	0.47	0.48	0.32	0.32	0.67	0.00	0.42	0.00	0.48	0.00	0.12

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

TABLE 4a
Mean Egg Weight (g) from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn) (Pre-Treatment)

	Pre-Treatment								
	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Total Weight	Mean
Non-transgenic Control Corn for Event 176 Hybrid									
Mean	55	53	54	57	54	55	55	310	54
SD	3	4	4	3	5	3	3	71	3
Transgenic Corn - Event 176-Derived Hybrid									
Mean	50	50	52	54	54	55	55	301	53
SD	5	3	3	5	4	3	3	72	3
Non-transgenic Control Corn for Event Bt11 Hybrid									
Mean	50	53	53	53	54	55	51	279	51
SD	3	4	5	2	5	6	9	93	6
Transgenic Corn - Event Bt11-Derived Hybrid									
Mean	53	52	54	54	54	52	55	306	54
SD	5	4	5	5	6	4	6	87	4

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

TABLE 4b
Mean Egg Weight (g) from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn) (Exposure)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Total Weight	Mean
Non-transgenic Control Corn for Event 176 Hybrid																
Mean	56	55	54	55	56	55	56	56	57	56	55	56	55	55	664	55
SD	4	3	2	2	3	2	3	4	2	3	2	3	3	2	134	2
Transgenic Corn - Event 176-Derived Hybrid																
Mean	54	51	54	52	55	54	54	56	55	55	54	54	55	54	630	54
SD	3	1	3	3	4	2	3	4	3	3	3	3	5	3	185	2
Non-transgenic Control Corn for Event Bt11 Hybrid																
Mean	50	52	53	53	54	54	53	49	56	52	55	56	54	54	575	52
SD	6	3	4	5	5	2	5	9	4	5	4	7	6	8	269	6
Transgenic Corn - Event Bt11-Derived Hybrid																
Mean	57	55	54	54	53	57	56	55	56	55	57	55	55	55	714	55
SD	11	6	4	5	4	6	4	6	6	4	6	6	5	6	117	5

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX I
DIET FORMULATION
WILDLIFE INTERNATIONAL LTD. LAYER RATION[1]

INGREDIENTS	PERCENT (%)
Fine Corn Meal	64
Soy Bean Meal, 47.5% Protein	18
Wheat Midds	2
Fish Meal, 60% Protein	6
Meat Poultry Blend, 58% Protein	0.03
Eastman Calphos	1
Salt Bulk Ag Mixing	0.25
Ground Limestone 37%	8
Selenium 06%	0.07
Poultry TM	0.07
Grolay V2X	0.07
Cho CHL70%	0.01
Liquim 40%	0.15
SQ-810 Supersk 2000#	0.15

NUTRITIONAL COMPOSITION	AMOUNT
Arginine	1.1842
Calcium	3.6525
Chloride	0.2315
Choline	1022.1072
Copper	14.5204
Crude Fat	2.9422
Crude Fiber	1.9593
Crude Protein	17.4008
Iodine	1.7851
Linoleic	1.3669
Lysine	0.8537
Magnesium	0.2228
Manganese	131.9649
Mepoult	1278.0145
Methionine	0.3382
Meth & Cys	0.6539
PHOS30	0.4564
PHOS50	0.5181
Potassium	0.5467
Selenium	0.5625
Sodium	0.1911
Total Phosphorus	0.6728
Tryptophan	0.1953
Vitamin D	1.5000
Xantophyll	8.9600
Zinc	121.0340

[1]The guaranteed analysis is a minimum of 18% protein, a minimum of 3% crude fat and a maximum of 3.2% crude fiber.

APPENDIX II

DIET PREPARATION

Treatment	**Test Substance (kg)**	**Agway® Lay Mix® (kg)**	**Limestone (kg)**
Non-Transgenic Control for Event 176-Derived Hybrid	7040	3300	660
Non-Transgenic Control for Event Bt11-Derived Hybrid	7040	3300	660
Transgenic Corn - Event 176-Derived Hybrid	7040	3300	660
Transgenic Corn - Event Bt11-Derived Hybrid	7040	3300	660

The diets were prepared by weighing the appropriate amount of each ingredient, placing them in a Patterson Kelly twinshell bulk blender and mixing for approximately 20 minutes.

APPENDIX III
Body Weight (g) by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event 176 Hybrid

Pen	Day 0	Change Day 0-Day 7	Day 7	Change Day 7-Day 14	Day 14	Total Change
1	1321	-63	1258	-28	1230	-91
2	1447	27	1474	19	1493	46
3	1557	-40	1517	-84	1433	-124
4	1537	-9	1528	-62	1466	-71
5	1401	14	1415	-42	1373	-28
6	1372	1	1373	-33	1340	-32
7	1506	3	1509	-205	1304	-202
8	1534	1	1535	-84	1451	-83
9	1331	28	1359	-69	1290	-41
10	1505	-42	1463	-213	1250	-255
Mean	1451	-8	1443	-80	1363	-88
SD	89	31	91	74	94	88

Transgenic Corn - Event 176-Derived Hybrid

Pen	Day 0	Change Day 0-Day 7	Day 7	Change Day 7-Day 14	Day 14	Total Change
1	1299	64	1363	-64	1299	0
2	1716	-74	1642	-46	1596	-120
3	1560	-285	1275	150	1425	-135
4	1376	-28	1348	-24	1324	-52
5	1377	4	1381	-74	1307	-70
6	1309	31	1340	-37	1303	-6
7	1266	56	1322	6	1328	62
8	1284	45	1329	-38	1291	7
9	1357	-16	1341	-4	1337	-20
10	1406	-63	1343	41	1384	-22
Mean	1395	-27	1368	-9	1366	-36
SD	141	103	100	65	96	60

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX III

Body Weight (g) by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event Bt11 Hybrid

Pen	Day 0	Change Day 0-Day 7	Day 7	Change Day 7-Day 14	Day 14	Total Change
1	1124	109	1233	-47	1186	62
2	1491	20	1511	-36	1475	-16
3	1369	5	1374	-56	1318	-51
4	1286	89	1375	-107	1268	-18
5	1564	1	1565	-52	1513	-51
6	1231	187	1418	1	1419	188
7	1370	7	1377	-56	1321	-49
8	1362	-118	1244	33	1277	-85
9	1367	-17	1350	-43	1307	-60
10	1638	-24	1614	-28	1586	-52
Mean	1380	26	1406	-39	1367	-13
SD	153	84	126	37	126	81

Transgenic Corn - Event Bt11-Derived Hybrid

Pen	Day 0	Change Day 0-Day 7	Day 7	Change Day 7-Day 14	Day 14	Total Change
1	1259	-20	1239	-26	1213	-46
2	1352	50	1402	-81	1321	-31
3	1461	-29	1432	-46	1386	-75
4	1392	-32	1360	-28	1332	-60
5	1394	11	1405	40	1445	51
6	1641	-92	1549	-24	1525	-116
7	1344	-100	1244	-5	1239	-105
8	1523	9	1532	-14	1518	-5
9	1425	-42	1383	-26	1357	-68
10	1475	106	1581	-12	1569	94
Mean	1427	-14	1413	-22	1391	-36
SD	106	62	117	31	122	67

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX IV

Feed Consumption (g/bird/day) by Pen from an Evaluation of Laying Hens (*Gallus gallus*) Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event 176 Hybrid
Pre-Treatment

Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Mean
1	139	194	136	161	111	110	82	133
2	140	131	101	133	106	79	75	109
3	193	149	140	133	126	115	102	137
4	150	136	101	175	103	97	96	123
5	145	94	116	148	71	120	67	109
6	145	126	73	157	83	96	88	110
7	136	139	121	129	95	107	92	117
8	133	95	109	118	91	85	125	108
9	148	170	123	110	89	93	86	117
10	121	143	134	104	103	93	85	112
							Mean	117
							SD	10

Transgenic Corn - Event 176-Derived Hybrid
Pre-Treatment

Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Mean
1	129	125	98	122	93	82	74	103
2	160	149	137	144	132	99	109	133
3	155	153	99	126	103	98	69	115
4	135	127	109	96	95	107	96	109
5	167	137	96	117	102	90	91	114
6	123	128	95	124	73	109	59	102
7	142	148	124	125	86	90	82	114
8	102	108	100	117	92	92	69	97
9	174	136	100	119	85	100	54	110
10	119	99	145	120	81	118	77	108
							Mean	111
							SD	10

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

Feed Consumption (g/bird/day) by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event 176 Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Mean
1	3	47	52	75	111	111	98	87	75	119	101	100	107	104	85
2	124	91	110	109	125	95	87	100	115	111	124	105	98	104	107
3	126	98	59	120	108	87	105	142	126	122	126	88	73	79	104
4	83	93	80	109	121	110	123	102	97	104	122	110	106	99	104
5	49	59	53	136	99	54	123	64	81	95	70	90	90	133	85
6	100	93	87	103	99	105	120	93	125	127	112	113	118	106	107
7	110	107	106	112	116	109	105	107	98	108	12	22	25	60	86
8	96	96	101	115	102	109	117	88	106	82	128	114	91	108	104
9	92	83	102	111	114	97	103	79	90	105	93	90	89	75	95
10	85	81	78	54	81	79	89	24	58	24	36	38	36	50	58
														Mean	93
														SD	16

Transgenic Corn - Event 176-Derived Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Mean
1	77	78	98	116	95	105	94	82	101	116	123	125	89	94	100
2	82	94	113	131	113	109	137	124	129	120	156	150	107	86	118
3	7	3	16	39	47	56	73	22	31	52	98	150	112	165	62
4	91	85	95	113	96	97	82	65	67	103	101	112	97	114	94
5	88	93	80	108	98	96	127	112	107	125	94	88	88	98	100
6	89	111	77	122	93	114	138	86	107	132	119	132	105	109	110
7	103	91	101	100	113	83	91	86	118	119	99	94	102	114	101
8	61	63	96	96	89	86	115	77	98	99	104	92	95	113	92
9	0	43	59	82	94	99	130	89	121	115	112	119	93	93	89
10	78	71	107	112	146	81	86	86	100	119	115	100	109	96	100
														Mean	97
														SD	15

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX IV
Feed Consumption (g/bird/day) by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event Bt11 Hybrid
Pre-Treatment

Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Mean
1	121	83	79	107	87	80	88	92
2	158	179	134	134	118	108	91	132
3	122	110	108	99	96	91	81	101
4	149	157	118	161	104	90	76	122
5	164	168	118	133	96	95	106	126
6	129	124	91	110	88	72	0	88
7	132	142	101	106	89	90	91	107
8	133	185	102	153	115	87	85	123
9	125	152	108	143	82	127	89	118
10	142	151	118	137	91	103	66	115
							Mean	112
							SD	15

Transgenic Corn - Event Bt11-Derived Hybrid
Pre-Treatment

Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Mean
1	120	145	108	105	79	100	77	105
2	123	146	115	112	86	84	81	107
3	172	145	137	116	112	108	90	126
4	136	143	117	139	109	106	94	121
5	128	140	126	123	55	105	81	108
6	181	174	129	137	91	159	103	139
7	154	164	129	119	89	122	112	127
8	110	132	110	112	96	105	103	110
9	104	119	102	99	74	84	76	94
10	161	171	103	104	120	104	83	121
							Mean	116
							SD	13

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX IV
Feed Consumption (g/bird/day) by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event Bt11 Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Mean
1	92	59	63	121	80	105	130	91	110	132	115	93	74	95	97
2	109	84	72	123	110	100	131	90	112	143	113	124	111	122	110
3	64	70	87	99	94	110	112	85	91	96	99	98	90	107	93
4	46	92	76	114	102	82	153	70	97	130	96	101	96	96	97
5	103	92	106	165	62	49	211	102	139	114	114	123	98	73	111
6	11	71	83	97	95	92	114	65	82	74	114	99	82	98	84
7	80	87	88	91	107	105	105	75	87	79	109	107	93	113	95
8	0	52	57	77	86	96	79	38	79	60	96	85	78	93	70
9	111	86	79	84	110	88	123	97	108	122	110	104	115	106	103
10	74	60	80	88	91	82	118	87	107	148	102	124	113	109	99
														Mean	96
														SD	12

Transgenic Corn - Event Bt11-Derived Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Mean
1	55	28	79	73	76	87	104	67	59	112	86	90	94	105	80
2	85	93	80	118	64	108	120	80	107	118	115	99	103	105	100
3	88	97	85	135	82	112	134	90	102	139	111	102	90	96	105
4	120	106	115	123	106	81	112	27	54	84	102	103	102	123	97
5	73	73	58	138	69	106	105	90	105	120	124	106	86	110	97
6	96	108	120	148	73	118	108	144	113	119	113	127	92	94	112
7	70	87	93	78	86	93	147	74	96	97	109	107	108	101	96
8	99	94	110	105	111	89	120	103	106	118	118	111	104	105	107
9	15	59	60	81	75	82	96	60	108	96	99	102	99	94	80
10	81	92	84	144	104	105	122	64	126	121	124	97	115	94	105
														Mean	98
														SD	11

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX V

Egg Production by Pen from an Evaluation of Laying Hens (*Gallus gallus*) Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-Transgenic Control Corn for Event 176 Hybrid

Pre-Treatment

Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Sum	E/H/D
1	1	1	1	1	1	1	0	6	0.86
2	0	1	1	1	0	1	1	5	0.71
3	1	1	1	1	1	1	1	7	1.00
4	1	0	1	1	1	1	1	6	0.86
5	0	1	1	0	1	1	1	5	0.71
6	0	1	1	1	1	1	1	6	0.86
7	0	1	1	1	1	1	2	7	1.00
8	0	0	0	0	1	1	1	3	0.43
9	1	1	1	1	1	1	1	7	1.00
10	0	1	1	1	1	1	1	6	0.86
							Mean	6	0.83
							SD	1	0.18

Transgenic Corn - Event 176-Derived Hybrid

Pre-Treatment

Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Sum	E/H/D
1	0	1	1	1	1	1	1	6	0.86
2	1	1	1	1	0	1	1	6	0.86
3	1	1	1	1	1	1	1	7	1.00
4	1	1	1	1	1	1	1	7	1.00
5	1	0	1	1	1	0	1	5	0.71
6	1	0	1	1	1	0	1	5	0.71
7	1	0	2	0	2	1	1	7	1.00
8	1	0	0	0	0	1	1	3	0.43
9	0	1	1	1	1	1	1	6	0.86
10	0	1	1	1	1	1	1	6	0.86
							Mean	6	0.83
							SD	1	0.18

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX V
Egg Production by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-Transgenic Control Corn for Event 176 Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Sum	E/H/D
1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	12	0.86
2	1	1	1	0	1	1	1	1	1	1	1	1	1	0	12	0.86
3	1	1	1	1	1	1	1	0	1	1	1	2	0	2	14	1.00
4	0	1	1	2	1	1	1	1	1	1	1	1	1	1	14	1.00
5	0	1	1	1	0	1	0	0	1	1	1	0	1	1	9	0.64
6	0	1	1	1	1	1	1	1	0	1	1	1	1	2	13	0.93
7	1	1	1	1	1	1	1	1	1	1	1	1	1	0	13	0.93
8	1	1	1	1	1	1	1	0	1	1	1	0	1	1	12	0.86
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	1.00
10	0	1	1	1	1	1	0	1	0	1	0	0	0	0	7	0.50
														Mean	12	0.86
														SD	2	0.16

Transgenic Corn - Event 176-Derived Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Sum	E/H/D
1	1	0	1	1	1	1	1	0	0	1	1	1	0	2	11	0.79
2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	15	1.07
3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0.14
4	1	1	0	1	1	1	1	1	1	1	1	1	0	2	13	0.93
5	1	1	2	1	1	1	1	1	1	1	0	1	1	1	14	1.00
6	2	0	1	1	1	1	1	0	1	1	1	1	1	1	13	0.93
7	1	1	1	0	1	1	1	1	1	1	1	1	0	1	12	0.86
8	1	1	1	1	0	1	1	1	1	1	0	1	1	2	13	0.93
9	0	1	1	1	1	1	1	0	1	1	1	1	1	1	12	0.86
10	0	1	1	1	0	1	1	1	0	1	1	1	1	1	11	0.79
														Mean	12	0.83
														SD	4	0.26

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX V

Egg Production by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-Transgenic Control Corn for Event Bt11 Hybrid
Pre-Treatment

Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Sum	E/H/D
1	1	0	0	0	0	0	1	2	0.29
2	1	0	1	1	1	1	1	6	0.86
3	1	0	1	1	0	1	1	5	0.71
4	1	1	0	1	1	1	1	6	0.86
5	1	1	1	0	1	1	0	5	0.71
6	1	0	1	1	1	0	0	4	0.57
7	1	1	1	1	1	0	1	6	0.86
8	1	1	1	1	0	1	2	7	1.00
9	0	1	1	0	1	1	1	5	0.71
10	1	1	1	1	1	1	1	7	1.00
							Mean	5	0.76
							SD	1	0.21

Transgenic Corn - Event Bt11-Derived Hybrid
Pre-Treatment

Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Sum	E/H/D
1	0	1	1	1	1	1	1	6	0.86
2	1	2	0	1	0	1	1	6	0.86
3	1	1	1	1	1	1	1	7	1.00
4	0	0	0	0	1	0	1	2	0.29
5	0	0	1	1	0	1	1	4	0.57
6	1	1	1	1	1	0	1	6	0.86
7	1	1	1	1	1	0	1	6	0.86
8	1	1	1	1	1	1	1	7	1.00
9	1	1	1	1	0	1	1	6	0.86
10	1	1	1	1	1	1	1	7	1.00
							Mean	6	0.81
							SD	2	0.22

(shaded) = Soft Shelled Egg

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX V

Egg Production by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-Transgenic Control Corn for Event Bt11 Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Sum	E/H/D
1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	12	0.86
2	1	1	0	1	1	0	1	1	1	1	1	1	1	1	12	0.86
3	1	1	1	1	1	1	1	1	1	1	1	1	1	2	15	1.07
4	1	1	1	1	2	1	1	0	1	1	1	1	1	1	14	1.00
5	1	1	1	1	1	0	1	1	1	1	0	1	1	1	12	0.86
6	0	0	0	0	0	0	0	1	0	1	1	0	1	1	5	0.36
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	1.00
8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0.07
9	0	1	1	1	1	1	1	1	1	0	1	1	1	1	12	0.86
10	0	1	1	1	1	1	2	1	1	0	1	1	1	1	13	0.93
														Mean	11	0.79
														SD	4	0.32

Transgenic Corn - Event Bt11-Derived Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Sum	E/H/D
1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	12	0.86
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	1.00
3	2	1	1	1	1	1	1	0	2	1	1	1	1	1	15	1.07
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	1.00
5	1	0	1	1	0	1	1	1	0	1	0	1	0	1	9	0.64
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	1.00
7	1	1	1	1	2	1	1	1	0	1	1	1	1	1	14	1.00
8	1	0	1	1	1	0	1	1	2	1	1	1	1	1	13	0.93
9	1	1	1	1	1	0	1	1	1	1	1	1	0	1	12	0.86
10	1	1	1	1	1	0	1	1	1	1	0	1	1	1	12	0.86
														Mean	13	0.92
														SD	2	0.12

= Soft Shelled Egg

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX VI

Egg Weight (g) by Pen from an Evaluation of Laying Hens (*Gallus gallus*) Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event 176 Hybrid

	Pre-Treatment							Total	
Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Weight	Mean
1	57	57	61	60	60	59	-----	354	59
2	-----	57	54	59	-----	56	57	283	57
3	54	53	54	56	56	57	58	388	55
4	50	-----	51	58	54	53	53	319	53
5	-----	47	49	-----	47	57	52	252	50
6	-----	48	50	58	53	56	53	318	53
7	-----	55	55	54	56	-----	55/54	329	55
8	-----	-----	-----	-----	45	51	50	146	49
9	57	55	56	55	57	48	60	388	55
10	-----	51	53	52	60	56	53	325	54
							Mean	310	54
							SD	71	3

Transgenic Corn - Event 176-Derived Hybrid

	Pre-Treatment							Total	
Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Weight	Mean
1	-----	48	50	51	52	54	54	309	52
2	51	53	55	53	57	55	55	379	54
3	54	54	56	61	60	59	59	403	58
4	49	49	49	50	-----	54	53	304	51
5	56	-----	54	60	53	-----	55	278	56
6	53	-----	51	52	53	-----	60	269	54
7	43	-----	49/50		54/50	55	56	357	51
8	46	-----	-----	-----	-----	52	50	148	49
9	-----	51	50	51	50	51	50	303	51
10	-----	47	54	-----	51	57	54	263	53
							Mean	301	53
							SD	72	3

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX VI

Egg Weight (g) by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event 176 Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Total Weight	Mean
1	61	59	58	57	57	56	-----	57	58	64	59	-----	55	55	696	58
2	59	57	56	-----	58	58	57	63	58	58	59	61	61	-----	705	59
3	59	59	56	58	55	56	57	-----	57	55	54	56/53	-----	54/54	783	56
4	-----	51	51	53/51	50	51	54	55	55	54	54	55	55	54	743	53
5	-----	52	53	53	-----	52	-----	-----	54	56	52	-----	52	59	483	54
6	-----	52	52	53	59	54	52	51	-----	53	53	52	54	57/54	696	54
7	52	54	54	55	55	55	52	56	57	57	55	54	54	-----	710	55
8	51	54	54	55	57	56	56	-----	56	56	55	-----	54	53	657	55
9	54	57	57	58	60	58	62	57	58	54	56	57	54	55	797	57
10	-----	53	51	53	56	54	-----	52	-----	54	-----	-----	-----	-----	373	53
														Mean	664	55
														SD	134	2

Transgenic Corn - Event 176-Derived Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Total Weight	Mean
1	52	-----	57	50	50	53	55	-----	-----	49	50	50	-----	50/49	565	51
2	56	57/56	56	57	59	56	61	59	60	61	60	60	60	57	875	58
3	60	51	53	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	164	55
4	52	51	-----	52	53	51	51	51	53	57	54	53	-----	53/54	685	53
5	52	51	51/55	53	53	55	53	57	57	55	-----	54	52	51	749	54
6	54/52	-----	52	51	60	55	53	-----	54	53	53	53	53	53	696	54
7	55	51	55	-----	55	55	54	52	54	55	54	54	-----	55	649	54
8	50	50	51	53	-----	53	57	58	55	55	-----	54	53	55/54	698	54
9	-----	52	51	49	53	52	51	-----	50	51	51	50	48	51	609	51
10	-----	53	57	54	-----	54	54	59	-----	56	53	55	61	56	612	56
														Mean	630	54
														SD	185	2

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX VI

Egg Weight (g) by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event Bt11 Hybrid

	Pre-Treatment							Total	
Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Weight	Mean
1	44	-----	-----	-----	-----	-----	31	75	38
2	54	-----	49	52	50	50	51	306	51
3	53	-----	53	51	-----	50	51	258	52
4	49	57	-----	53	48	48	51	306	51
5	52	53	59	-----	58	60	-----	282	56
6	46	-----	52	52	49	-----	-----	199	50
7	51	50	50	52	56	-----	54	313	52
8	50	58	52	56	-----	53	55/56	380	54
9	-----	48	46	-----	54	56	57	261	52
10	52	53	61	57	61	65	59	408	58
							Mean	279	51
							SD	93	6

Transgenic Corn - Event Bt11-Derived Hybrid

	Pre-Treatment							Total	
Pen	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Weight	Mean
1	-----	45	45	45	46	48	48	277	46
2	49	50/58	56	55	-----	55	53	376	54
3	49	52	53	52	57	56	57	376	54
4	-----	-----	-----	-----	52	-----	55	107	54
5	-----	-----	52	53	-----	54	57	216	54
6	54	54	61	61	59	-----	65	354	59
7	56	57	58	60	63	-----	62	356	59
8	50	53	53	53	53	52	52	366	52
9	62	56	57	55	-----	54	54	338	56
10	52	49	50	-----	49	46	46	292	49
							Mean	306	54
							SD	87	4

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX VI

Egg Weight (g) by Pen from an Evaluation of Laying Hens (*Gallus gallus*)
Fed Diets Containing Transgenic Event 176 and Bt 11 Maize (Corn)

Non-transgenic Control Corn for Event Bt11 Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Total Weight	Mean
1	41	-----	46	44	46	51	45	41	-----	48	50	48	46	47	553	46
2	51	53	-----	53	51	-----	50	50	52	53	51	56	51	52	623	52
3	49	49	51	53	50	52	51	51	52	52	53	51	50	51/51	766	51
4	49	48	51	50	52/53	54	56	-----	55	55	55	55	53	53	739	53
5	58	56	58	62	59	-----	59	56	62	59	-----	59	57	57	702	59
6	-----	-----	-----	-----	-----	-----	-----	31	-----	42	-----	-----	-----	40	113	38
7	53	52	54	52	56	53	56	53	51	53	60	55	54	59	761	54
8	-----	54	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	54	54
9	-----	52	52	51	59	56	57	53	57	-----	55	55	55	55	657	55
10	-----	55	56	57	56	55	55/59	58	60	-----	60	72	68	66	777	60
														Mean	575	52
														SD	269	6

Transgenic Corn - Event Bt11-Derived Hybrid

Pen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Total Weight	Mean
1	47	48	50	48	48	47	-----	48	49	54	49	47	-----	46	581	48
2	53	54	54	54	54	53	52	53	53	53	54	53	53	54	747	53
3	56/54	56	56	57	54	56	58	-----	55/57	55	56	55	56	58	839	56
4	55	56	53	54	59	59	53	55	58	54	66	57	55	55	789	56
5	86	-----	57	60	-----	59	61	63	-----	59	-----	63	-----	64	572	64
6	56	66	61	60	59	62	59	66	66	62	63	64	63	64	871	62
7	58	59	57	58	82/50	64	62	57	-----	58	59	59	58	57	838	60
8	53	-----	51	51	51	-----	52	52	52/51	51	52	52	52	54	674	52
9	56	51	53	54	53	-----	54	53	59	53	53	53	-----	53	645	54
10	49	50	47	45	48	-----	50	52	53	47	-----	47	48	47	583	49
														Mean	714	55
														SD	117	5

The above differences between the treatment groups and their corresponding control groups were not statistically significant ($p \leq 0.05$).

APPENDIX VII

ANALYSIS OF TISSUE SAMPLES FOR 14-DAY POULTRY STUDY OF BT11 AND EVENT 176-DERIVED MAIZE

REPORT NO. NSB-005-97

NOVARTIS SEEDS, INC.
PRODUCT REGISTRATION LABORATORY
SEEDS BIOTECHNOLOGY RESEARCH UNIT
RESEARCH TRIANGLE PARK, NC USA

SUMMARY

Laying hens were fed for 14 days with feed formulated from grain derived from either Event 176 or Event Bt11 *Bt* maize (corn) plants, or from control maize plants. Tissue samples (white meat, dark meat, liver, egg white, and egg yolk) from these hens were analyzed for the presence of the transgenic proteins, CryIA(b) and PAT. Neither protein was detected in any tissue analyzed.

INTRODUCTION

Grain from Novartis Seeds' Event 176- and Bt11-derived transgenic maize plants and corresponding control maize plants was used to prepare feed for a 14-day poultry study entitled, "An Evaluation of Laying Hens (*Gallus gallus*) Fed Diets Containing Transgenic Event 176 and Bt11 Maize (Corn)," conducted at Wildlife International Ltd., Easton, MD, USA (Wildlife), from 12 August 97 to 26 August 97. The objective of this study was to evaluate the general health and egg production parameters of laying hens fed diets prepared with grain from either transgenic maize plants or the corresponding control maize plants, and to evaluate eggs and selected tissues for the presence of the transgenic proteins, CryIA(b) and phosphinothricin acetyltransferase (PAT).

In support of the 14-day poultry study conducted at Wildlife, Novartis Seeds Product Registration Laboratory (Novartis Seeds Biotechnology Research Unit, Research Triangle Park, NC) conducted all necessary analyses of grain, feed, and animal tissue samples. The animal tissue samples analyzed included white (breast) meat, dark (thigh) meat, liver, egg white and egg yolk. The results of these analyses are presented in this report.

MATERIALS AND METHODS

Source of grain. All grain used to prepare the poultry feeds was harvested from field-grown plants in 1996. The Bt11 and the corresponding nontransgenic control grain were from hybrids N4640Bt and N4640, respectively, grown in Stanton, MN. The Event 176 and control grain was from hybrids MAX454 and G4494, respectively, grown in Bloomington, IL. All grain was stored in either an unheated warehouse during the winter months or within a cold room. Temperatures during storage did not exceed 24^{o}C.

Source of feed. Samples of poultry feed were formulated at Wildlife as described in the Diet Preparation section of Wildlife's in-life report (project number 108-394). On Day 0 of the study, Wildlife personnel collected 50 g samples of the four ground grain types and 50 g samples of each of the four formulated feeds. These were shipped overnight on dry ice to Novartis Seeds Product Registration Laboratory where they were received on 14 August 97. On Day 7, 50 g samples of each formulated feed were also collected and shipped overnight on dry ice to Novartis Seeds Product Registration Laboratory where they were received on 22 August 97. All samples were analyzed for the presence of CryIA(b) and PAT as described below.

Source of tissue. On Days 13 and 14 of the study, eggs were collected and separated into whites and yolks, and Day 14, study termination, samples of white (breast) and dark (thigh) meat, as well as the liver were collected. All samples were frozen on dry ice and shipped overnight on dry ice to Novartis Seeds Product Registration Laboratory where they were received on 28 August 97. Representative samples of white and dark meat, liver, egg yolk and egg white were analyzed for the presence of CryIA(b) and PAT as described below.

Extraction and analysis. A total of 272 (4 grain, 8 feed and 260 tissue) samples were shipped from Wildlife to Novartis Seeds for analysis. All grain and feed samples, as well as 55 representative tissue samples, were analyzed for the presence of the transgenic proteins, CryIA(b) and PAT. The tissue samples included 5 out of 18 and 5 out of 17 egg (white and yolk) samples from treatment groups that received feed prepared from Event 176-derived and Bt11-derived grain, and 5 out of 10 samples for liver, breast and thigh meat from each of these treatment groups. One control sample from the nontransgenic Event 176-derived hybrid treatment group was also analyzed for each of the five tissues. No control samples from the nontransgenic Bt11-derived hybrid treatment group were analyzed (See Results section).

All feed and tissue samples were prepared and extracted in accordance with SOP 2.7. Sample extracts were quantitatively analyzed for CryIA(b) protein and PAT protein by enzyme-linked immunosorbent assays (ELISA) (SOPs 2.2 and 2.29, respectively). The lower limit of quantitation of the double sandwich ELISA for CryIA(b) protein was approx. 6 ng/g tissue (6 ppb) and for PAT was approx. 30 ng/g tissue (30 ppb). The proteins were considered not detectable if the mean absorbance generated by ELISA did not exceed that of the negative control.

RESULTS

In Bt11-derived grain, the level of CryIA(b) was found to be 384.2 ng/g fresh weight. The feed prepared with Bt11-derived grain measured 240.1 and 262.5 ng/g fresh weight on Day 0 and Day 7, respectively, reflecting that the feed contained 64% grain (Table 1). Analysis of the grain feed samples from hybrid N4640 (the nontransgenic controls for Bt11-derived plants) revealed low but quantifiable levels of CryIA(b), indicating that this grain was contaminated with transgenic kernels. Because the birds in this group received transgenic proteins in their diet, this group was no longer considered a control group, and tissue samples taken from the birds fed this feed were excluded from further analysis in the study. As reported previously (Fearing *et al*., 1997), only trace levels of CryIA(b) are found in Event 176-derived grain and therefore it was not unexpected that no CryIA(b) was detected in the feed produced from this grain (Table 1). (Analyses completed 26 Aug. 97, Notebook # SeBRU-72, pp. 5 – 20, 47.)

Trace amounts of PAT protein were found in Event 176-derived grain; however, it was not detected in the corresponding feed (Table 2). In Bt11-derived grain, the level of PAT protein was found to be 73.5 ng/g fresh weight. PAT levels were determined to be 58.8 ng/g fresh weight in feed formulated with Bt11-derived grain on Day 0, and trace amounts were detected in the Day 7 feed sample (Table 2). (Analyses completed 26 Aug. 97, Notebook #SeBRU-72, pp. 5 – 20, 47.)

Analysis of the selected tissue samples revealed no detectable CryIA(b) or PAT protein in any sample (Table 3). (Analyses completed 10 Sept. 97, Notebook #SeBRU-72, pp. 21 – 46.)
The remaining samples are stored at -80°C at the Novartis Seeds Biotechnology Research Unit, Research Triangle Park, North Carolina.

CONCLUSION

As expected (Fearing *et al.*, 1997), the Event 176-derived grain contained only trace levels of CryIA(b) protein and no detectable PAT protein. The feed formulated from this grain contained no detectable levels of either transgenic protein. CryIA(b) and PAT protein were found in both the grain from the Event Bt11-derived hybrid and feed formulated from this grain. Neither transgenic protein was detected in any animal tissue sample analyzed.

RECORDS RETENTION: Raw data, the original copy of this report, and other relevant records are archived at Novartis Seeds Biotechnology Research Unit, 3054 Cornwallis Road, Research Triangle Park, NC USA 27709.

CONTRIBUTING SCIENTISTS: Analytical work reported herein was conducted at the Novartis Seeds Product Registration Laboratory (Novartis Seeds Biotechnology Research Unit) by [redacted]

GLP COMPLIANCE: This study was not conducted in compliance with all the provisions of Good Laboratory Practice Standards (40 CFR 160, Federal Register, 1989). Of the applicable sections of 40 CFR §160, specific areas of non-compliance were §160.31 (c) and (g), §160.33 (a) (d) and (e), §160.35, §160.120, §160.130, §160.185 (a)(2) and (14) and §160.195 (d). However, the study was conducted according to accepted scientific procedures and SOPs, and all raw data and appropriate records were maintained.

Reported by: ____________________ ____________________
[redacted] Date

Approved by: ____________________ ____________________
[redacted] Date

References:

Standard Operating Procedures

SOP 2.2	Quantitative Analysis for CryIA(b) by ELISA
SOP 2.7	CryIA(b) Extraction from Maize Tissues and Silage
SOP 2.29	Quantitative Analysis for PAT by ELISA

Literature References

Fearing, P. L., Brown, D., Vlachos, D., Meghji, M., and Privalle, L. (1997) Quantitative analysis of CryIA(b) expression in Bt maize plants, tissues, and silage and stability of expression over successive generations. Mol. Breeding 3: 169-176.

Table 1. CryIA(b) Protein Levels in Poultry Feed Prepared from Transgenic *Bt* maize Grain and Non-transgenic Control Grain

Sample	**Transgenic Event 176-Derived Hybrid**	**Non-Transgenic Control for Event 176-Derived Hybrid**	**Transgenic Event Bt11-Derived Hybrid**	**Non-Transgenic Control for Event Bt11-Derived Hybrid**[a]
	(ng CryIA(b)/g fresh weight)			
Grain (Rec'd 8/14/97)	< 6[b]	0	384.2	20.6
Day 0 Feed (Rec'd 8/14/97)	ND[c]	0	240.1	17.6
Day 7 Feed (Rec'd 8/21/97)	ND	0	262.5	16.9

[a] Analysis revealed quantifiable levels of CryIA(b), indicating that the grain was contaminated with transgenic kernels.

[b] Where trace amounts were detectable but not quantifiable, values are shown as less than (<) the lower limit of quantitation.

[c] ND = not detectable – values obtained by ELISA did not exceed those of the control.

Table 2. PAT Protein Levels in Poultry Feed Prepared from Transgenic *Bt* maize Grain and Non-transgenic Control Grain

Sample	**Transgenic Event 176-Derived Hybrid**	**Non-Transgenic Control for Event 176-Derived Hybrid**	**Transgenic Event Bt11-Derived Hybrid**	**Non-Transgenic Control for Event Bt11-Derived Hybrid**[a]
	(ng PAT/g fresh weight)			
Grain (Rec'd 8/14/97)	< 30[b]	0	73.5	< 30
Day 0 Feed (Rec'd 8/14/97)	ND[c]	0	58.8	< 30
Day 7 Feed (Rec'd 8/21/97)	ND	0	< 30	ND

[a] Analysis revealed quantifiable levels of PAT, indicating that the grain was contaminated with transgenic kernels.

[b] Where trace amounts were detectable but not quantifiable, values are shown as less than (<) the lower limit of quantitation.

[c] ND = not detectable – values obtained by ELISA did not exceed those of the control.

Table 3. CryIA(b) and PAT Levels in Various Tissues of Laying Hens Fed on Diet Formulated with Grain Derived from Transgenic Bt Hybrids[a]

Sample Number	Non-Transgenic Control Diet	Sample Number	Event 176-Derived Diet	Sample Number	Bt11-Derived Diet
White Meat Samples					
1	ND[b]	61	ND	91	ND
		67	ND	97	ND
		73	ND	103	ND
		79	ND	109	ND
		85	ND	115	ND
Dark Meat Samples					
2	ND	62	ND	92	ND
		68	ND	98	ND
		74	ND	104	ND
		80	ND	110	ND
		86	ND	116	ND
Liver Samples					
3	ND	63	ND	93	ND
		69	ND	99	ND
		75	ND	105	ND
		81	ND	111	ND
		87	ND	117	ND
Egg White Samples					
9	ND	33	ND	45	ND
		35	ND	47	ND
		37	ND	49	ND
		39	ND	51	ND
		41	ND	53	ND
Egg Yolk Samples					
10	ND	34	ND	46	ND
		36	ND	48	ND
		38	ND	50	ND
		40	ND	52	ND
		42	ND	54	ND

[a] The lower limit of quantitation for the CryIA(b) and PAT ELISA are 6 and 30 ng/g tissue, respectively.
[b] ND = not detectable – values obtained by ELISA did not exceed those of the control.

APPENDIX VIII

PERSONNEL INVOLVED IN STUDY

The following key personnel were involved in the conduct or management of this study:

<u>Avian Toxicology</u>

[redacted]

Application for renewal of authorization of Bt11 maize and derived products notified according to Articles 11 and 23 of Regulation (EC) No 1829/2003 on genetically modified food and feed

PART I: TECHNICAL DOSSIER

APPENDIX 8.2

Evaluation of transgenic event 176-Derived and Bt11-Derived Bt corn (maize) in the diet of lactating dairy cows

FINAL REPORT

TITLE: **Evaluation of Transgenic Event 176-Derived and Bt11 –Derived "Bt" Com (Maize) in the Diet of Lactating Dairy Cows**

Starting Date of Experiment: 18 September 1996

Completion Date of Experiment: 2 October 1996

Reported by: [redacted]

15 Oct. 1997
Date

Associate Professor of Animal Science

Address:
Department of Animal Science
123 Kildee Hall
Iowa State University
Ames, Iowa 50011 USA

Site Location:
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Sponsor:
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Abstract

A 14 day study was conducted to determine whether milk produced by lactating dairy cows contained CryIA(b) or PAT proteins when cows are fed fresh whole-plant corn from Novartis Seeds' transgenic *Bt* hybrids. Twelve high producing Holstein dairy cows were selected from the Iowa State University dairy herd in Ames, Iowa, USA, and were assigned to receive one of 3 study diets; study diets contained chopped whole-plant corn from commercial Novartis Seeds hybrids representing 1) an Event 176-derived transgenic hybrid, 2) a Bt11-derived transgenic hybrid, or 3) a non-transgenic control hybrid. Daily composite milk samples from each cow and four additional composite milk samples to which purified CryIA(b) and PAT proteins were added intentionally were evaluated by the Seeds Biotechnology Research Unit of Novartis Seeds, Research Triangle Park, NC, USA for the presence of CryIA(b) and PAT proteins. A second set of composite milk samples was evaluated by Dairy Lab Services in Dubuque, Iowa, USA for milk components. The CryIA(b) protein was detected in samples of diet that were prepared using fresh chopped whole-plant corn from the two transgenic hybrids, but no CryIA(b) protein was detected in samples of the control study diet. No CryIA(b) or PAT proteins were detected in standard samples of milk collected from cows fed study diets, however, transgenic proteins were detected in all 55 samples to which CryIA(b) and PAT proteins were added intentionally. On average, groups of cows that were fed study diets consumed more than 43 kg of feed (as fed) daily per cow and produced more than 38 kg of milk daily per cow. Milk yield, feed intake, composition of milk, and udder health were similar for all study diet groups. In general, cows remained healthy and in good body condition throughout the study. Findings from this study indicate clearly that CryIA(b) and PAT proteins cannot be detected in milk from cows that are consuming diets prepared using Novartis Seeds' transgenic corn hybrids that produce these proteins. Moreover, we detected no adverse effects of these transgenic proteins for dairy cows that consumed green plant material from Novartis Seeds' transgenic *Bt* corn hybrids.

Introduction

Transgenic, insect-protected corn (maize) is a relatively small but increasing proportion of the field corn that is grown in the US today. Transgenic commercial hybrids that resist damage and prevent yield losses caused by the European corn borer (*Ostrinia nubilalis* Hübner), a devastating insect pest of corn in North America and Europe, are commonly referred to as "*Bt*" corn, and have been developed using tools of modern plant biotechnology. Most *Bt* corn hybrids that are grown currently in the US produce a CryIA(b) insecticidal protein that is similar to an insecticidal protein occurring naturally in the common soil microbe, *Bacillus thuringiensis*.

The CryIA(b) protein is a member of a class of insecticidal proteins that are produced as parasporal crystals (hence, the name "Cry") by *Bacillus thuringiensis*. The CryIA(b) protein is toxic when ingested by specific lepidopteran (caterpillar) pests, because receptor-like sites for the protein are present in the midgut membrane of larvae from these susceptible species. In susceptible species, the CryIA(b) protein binds to these sites, consequently, membrane integrity of the midgut is disrupted and the larvae cease feeding and die. Other organisms lack gut binding sites that "recognize" CryIA(b). For mammals, birds, beneficial insects, fish and other wildlife, CryIA(b) is non-toxic and is digested by these species as an ordinary dietary protein. In the US, *Bacillus thuringiensis*-based insecticides that contain multiple Cry proteins have been registered and approved for foliar use on food crops since 1961 (US Environmental Protection Agency, 1986).

In addition to CryIA(b), Event 176- (Koziel et al., 1993) and Bt11-derived corn plants produce the enzyme phosphinothricin acetyltransferase (PAT). This marker protein was used during the original gene transfer events to identify or select plants that were transgenic. Recently, safety for the CryIA(b) and PAT proteins were assessed, and *Bt* hybrids derived from the corn transformation experiment "Event 176" (developed by the former Ciba Seeds) and the event designated "Bt11" (developed by the former Northrup King Co.) were approved for all uses in the US (US Environmental Protection Agency, 1997a; 1997b). Commercial corn hybrids derived from these two transgenic events are currently marketed by Novartis Seeds, Inc. (formed by the merger of Ciba Seeds and Northrup King).

Under economic conditions in the US, it is necessary for dairy cows (*Bos taurus*) to produce large quantities of milk, and thus, for lactating cows to consume large quantities of feed. Roughages or forages constitute a large portion of this diet for dairy cows, and whole-plant corn ("green chop") is used frequently as a roughage source in commercial rations for dairy cows. Dairy cows likely would consume more *Bt* corn plant material than other species. Safety reviews conducted by the US Environmental Protection Agency, US Department of Agriculture, and the US Food and Drug

Administration identified no risk for mammalian safety by feeding Event 176- and Bt11-derived corn, however, to confirm that there were no unexpected effects for milk composition and dairy cow health from consuming *Bt* corn, a feeding study was designed and conducted for lactating dairy cattle.

For this study, lactating dairy cows were fed diets containing fresh whole-plant corn from commercial Novartis Seeds varieties representing 1) a transgenic Event 176-derived hybrid, 2) a transgenic Bt11-derived hybrid, or 3) a conventional, nontransgenic control hybrid representing the same genetic background as the Event 176-derived hybrid. Although measurable levels of CryIA(b) protein are detectable in fresh, green corn plants, the process of ensiling (fermentation process that is conducted under conditions of limited oxygen) has been shown to rapidly degrade CryIA(b) in plant material (Fearing et al., 1997). Typically, ensiled whole-plant corn is fed to dairy cows, however, to maximize the potential for exposure to the transgenic proteins in *Bt* corn, cows received no ensiled feedstuffs, and fresh whole-plant corn constituted the sole roughage source for the prepared feed.

As part of this evaluation, milk was collected from cows fed the study diets, and analyzed for the presence of CryIA(b) and PAT proteins. In general, compounds that are ingested by lactating cows and are absorbed intact into the blood can be detected in milk within several hours after ingestion, however, milk residue studies that are conducted for the Food and Drug Administration in the US typically require a feeding period of one or more weeks to ensure that no residues are evident after sustained feeding. To determine whether CryIA(b) insecticidal and PAT proteins are present in milk after sustained feeding of fresh whole-plant *Bt* corn, samples of milk were collected during a two week period for this study. Also, to confirm that the analytical methods and sample handling procedures used could allow for objective detection of the transgenic proteins by laboratory personnel, predetermined quantities of purified CryIA(b) and PAT proteins were intentionally introduced into additional samples of milk after collection. Laboratory personnel who evaluated the samples were unaware of the identification for samples that contained these added, purified proteins, and these samples were analyzed simultaneously with the routine milk samples collected throughout the study.

Materials and Methods

<u>Source of fresh whole-plant corn</u> Novartis Seeds transgenic corn hybrids derived from Event 176 (MAX21) and Event Bt11 (N6800Bt) and a non-transgenic commercial control hybrid (Ciba 4365)[1] were used as the source of whole-plant corn. Fresh corn plants were harvested, chopped, and

[1] Ciba 4365 is the conventional, isogenic counterpart for the MAX21 hybrid.

used for preparing diets daily. The practice of daily harvesting is used commercially when fresh forages (green chop) are fed to dairy cows. In addition, daily harvest of corn plants was necessary to ensure that the feed fed to cows contained the highest levels possible of the transgenic proteins, and that the opportunity for degradation of these proteins in feedstuffs was minimized.

Corn plants were chopped using the wood chipping attachment of a stationary wood chipper/chopper to provide a feedstuff that was suitable for healthy rumen function in high producing lactating dairy cows. Stationary wood chipper/chopper units frequently are used to process corn plant material when relatively small quantities of a feedstuff are needed for research studies.

All corn used for this study was field-grown within a 10 mile (16.1 km) radius of Ames, Iowa, USA using standard local agronomic practices. For a given day, plants were harvested by the same personnel, thus, all corn was subjected to similar environmental and handling conditions. Each day of the study, whole-plant corn was harvested in the morning; chopped fresh whole-plant corn was used to prepare diets immediately prior to the 6:00 PM feeding. On 18 September 1996, Personnel from the Seeds Biotechnology Research Unit of Novartis Seeds (Research Triangle Park, NC, USA) conducted preliminary analyses by ELISA (see Materials and Methods) of fresh chopped corn plant material and respective diets that were prepared using the fresh chopped corn. These analyses confirmed the presence of the CryIA(b) insecticidal protein in fresh corn plant materials and diets from transgenic corn hybrids (Table 1), however, PAT protein was not detected in these samples. The absence of detectable levels of PAT protein in the whole-plant corn may be attributable to instability of the protein in harvested plant material of the absence of significant levels of PAT in mature plants.

Corn plants derived from the control variety do not produce the transgenic proteins, thus it was expected that samples of fresh chopped whole-plant corn and diet prepared using plants from this variety would contain no CryIA(b) insecticidal proteins. However, a level of 66.4 ng CryIA(b) per g of fresh chopped whole-plant corn was reported for the sample from this variety that was submitted for analyses (Table 1). No CryIA(b) insecticidal protein was detected in diet prepared on 18 September 1996 using corn plants from this control variety (Table 1); also, no transgenic proteins were reported for samples of fresh control corn and the corresponding prepared diet that were collected during subsequent days of the study. Thus, it is likely that this sample of fresh corn from the control variety was contaminated inadvertently during sample collection on 18 September 1996 by corn plant material from one of the transgenic varieties included in the study, and no implications of this aberrant result are expected for interpreting findings from this study.

Experimental Design and Assignment of Cows to Study Diets The study consisted of feeding three different diets that had been prepared using fresh chopped whole-plant corn from Novartis Seeds hybrids: 1) Event 176 derived, 2) Bt11 derived, and 3) a non-transgenic control that was the isogenic counterpart for the Event 176 derived hybrid. Twelve lactating Holstein dairy cows in their second through fifth parity were selected from the Iowa State University dairy herd in Ames, Iowa, USA based on their current age, days since calving, and milk, fat, and protein production; four cows were assigned to each group that received one of the 3 study diets for the 14 days of the experiment. Cows that were in their first parity, had low milk production, were in late lactation (more than 200 days since calving), or exhibited any signs of health problems were not selected for this study.

The current body weight and production level of energy corrected milk[2] are known to influence the feed consumption and future milk production for cows, thus cows were assigned to groups so that average body weight and production of energy corrected milk were similar for the groups. Study group averages for several important production and body weight characteristics of cows when assigned to the groups are in Table 2.

Analyses of chopped whole-plant corn Samples of the chopped whole-plant corn from the first full day of study were collected and sent for proximate analyses to the Iowa Testing Laboratories, Inc. in Eagle Grove, Iowa, USA. Results for these analyses are in Table 3, and are represented on an “as is” or “as fed” basis. A large portion of the differences in reported composition among the three samples of chopped whole-plant corn likely is due to differences for moisture. Also, stage of maturity differs for different hybrids, fields, and areas within a field, thus, samples may have differed slightly for stage of maturity. These differences may have influenced results from proximate analyses.

In addition, varieties selected for this trial are known to differ for characteristics other than presence of the *Bt* transgene, thus differences in results from the proximate analyses may have been due to known differences that are inherent to these varieties. The relative degree of similarity for analytical values of control and Event 176 derived hybrids is consistent with the isogenicity of their germplasm. Compositional differences between the sources of chopped whole-plant corn were considered when diets were formulated, and thus, differences other than the presence of the CryIA(b) protein in plant tissues are expected to have

[2] Energy corrected milk is the total yield of milk that has been standardized to a constant energy content from milk, fat, and protein.

essentially no impact on milk, fat, and protein production of cows. Furthermore, compositional differences other than the presence or absence of CryIA(b) protein in the feedstuffs should be unimportant when determining whether milk produced by lactating dairy cows contains transgenic proteins when cows are fed fresh whole-plant corn from transgenic and non-transgenic hybrids.

Diet Formulation Study diets were formulated to meet or exceed the National Research Council allowances of energy, protein, vitamins, and minerals for maintenance (660 kg body weight) and daily milk and fat production (43 kg milk and 3.5% fat) of lactating dairy cows (National Research Council, 1988). It was assumed that cows would consume approximately 22.7 kg of dry matter from feed daily, and that dry hay would account for approximately 18% of the dry matter consumed. Rations for the study groups consisted of wet corn gluten feed[3], ground corn grain[3], whole cottonseed[3], roasted soybeans[3], vitamin and mineral premix, and the respective chopped whole-plant corn (Table 4). Moisture content of the chopped fresh whole-plant corn was monitored and rations reformulated when significant change in moisture content was detected.

The regular herd diet that was fed to cows prior to the start of the study included wet corn gluten feed, ground corn grain, whole cottonseed, roasted soybean meal (44% crude protein), vitamin and mineral premix, corn silage, and alfalfa silage (lucerne haylage). However, ensiled feedstuffs were excluded intentionally from the study diets, because findings from studies conducted previously by Novartis Seeds indicated that CryIA(b) protein is degraded rapidly in silage (Fearing et al., 1997). To decrease the possibility for digestive disorders that result when cows experience a radical change in diet, such as the replacement of ensiled feeds by fresh whole-plant corn and dry hay, changes in the diet were made incrementally during a 4 day acclimation period. For the 4 days of the acclimation period, chopped fresh whole-plant corn and dry hay replaced 20%, 40%, 60%, and 80%, respectively, of forages in the regular herd diet, thus, by day 5 of the study, cows were consuming their respective study diet.

Access to Feed and Water Cows were provided access to feed and fresh water at all times, except during milking. Each day, fresh feed was mixed for cows during the evening, and approximately 60% of this feed was dispensed to cows immediately following the 6:00 PM milking. The remaining 40% of the feed from each day’s mix was stored overnight in plastic bins and was dispensed to cows at approximately 7:00 AM. All feed

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[3] Feedstuffs were obtained from a feed supplier and were from commercial varieties.

that was not consumed by approximately 6:00 PM on the following day was removed from the feed bunk and was discarded. To ensure that feed was available to cows at all times, the total amount of feed prepared for each study group was adjusted regularly based on feed consumption during the previous 24 hour period. The objective was to make available to cows 20% more feed than they consumed. Fresh dry hay was made available to cows throughout the day.

Cows were housed in one tie-stall barn at the Iowa State University Dairy Farm in Ames, Iowa, USA. The stalls in this barn are 1.22 X 1.83 m and 1.22 X 1.98 m; large cows are housed in the 1.98 m long stalls. For this experiment, all study groups included cows housed in both sizes of stalls. To ensure comfort for cows and to provide a stall environment that minimizes exposure of the udder to environmental organisms that cause mastitis, the stall surface is covered with a layer of sand that is more than 20 cm deep. Cows were restrained in their respective stalls for the majority of the day, but were released for milking and routine daily exercise. All cows were fed twice daily, mornings and evenings, and were milked three times daily (2:00 AM, 10:00 AM, and 6:00 PM). Feed intake, milk production, and general appearance of cows were monitored regularly, and any abnormal changes were recorded. Veterinarians who are employed by the Iowa State University Veterinary Field Services were available at all times to provide any necessary medical care. Housing, management, and care for cows in this study did not differ from typical daily routines used in commercial dairy herds.

<u>Data Collection</u> Body weights for cows were recorded on the first and last days of the trial. Milk production data were collected for 8 days prior to the start of the study period, 4 days following the end of the study period, and daily throughout the study period, except for 21, 22, and 25 September 1996 when data were not available as a result of problems with the farm computer system. Throughout the study period, samples of milk were collected from each cow during all milking times. Using these individual milking samples, composite samples were created for cows and contained equal volumes of milk (2 ml) from each of the three daily milkings. Samples of chopped whole-plant corn and respective diets prepared using the corn were collected daily, also. Samples of corn, diet, and one set of composite milk samples were frozen and shipped overnight to the Seeds Biotechnology Research Unit of Novartis Seeds for analyses for the presence of CryIA(b) and PAT proteins. Samples were processed, extracted, and analyzed by enzyme-linked immunosorbent assays (ELISA) as described by Fearing et al. (1997). Lower limits of quantification for the ELISA were: for CryIA(b)

protein - 8 ng/g fresh weight of diet and fresh plant material and 3 ng/ml of milk, and for PAT protein - 200 ng/g fresh weight of diet and fresh plant material and 107 ng/ml of milk. A second set of composite milk samples were preserved using bromopol and were refrigerated until samples for approximately four days were shipped overnight to Dairy Lab Services in Dubuque, Iowa, USA for analyses of milk components. Any observed changes in clinical health for cows were noted, also.

To verify that CryIA(b) and PAT proteins can be detected when present in raw milk samples that have been frozen and shipped, four additional composite milk samples were prepared daily by adding purified CryIA(b) and PAT proteins intentionally. These four samples were prepared by using milk from cows that had been selected at random from the cows in the experiment, and contained concentrations of intentionally added purified CryIA(b) and PAT proteins of 1.8 and 0.5 μg/ml, respectively. Samples were packed and shipped with other samples for a given day, thus, identification for the samples containing intentionally added purified CryIA(b) and PAT proteins was unknown to the Seeds Biotechnology Research Unit of Novartis Seeds at the time of evaluation.

<u>Statistical Analyses</u> Data were analyzed using the mixed models procedure of SAS (1995). Critical probability value for statistical tests was 0.10. To determine whether groups differed when fed diets containing transgenic and non-transgenic chopped whole-plant corn, milk yield; change in body weight; feed intake; and levels in milk of CryIA(b) protein, PAT protein, milk fat, milk protein, lactose, solids-not-fat, other solids, total solids, and somatic cell count were evaluated. The somatic cell count in milk is an indicator of health status for the udder, and high somatic cell counts (a typical threshold level is $>$ 500,000 cells/ml) are associated with mastitis.

Fixed independent variables of statistical models for milk yield, somatic cell count, feed intake, milk components, and levels of CryIA(b) and PAT proteins were study diet (3 diets), parity (3 levels - parities 2, 3, and 4 & 5), sample collection date, and days since calving (continuous effect); cow within a combination of diet and parity and residual were random independent variables. The cow within diet $\times$ parity term was used to test for the importance of study diet. When change in body weight was evaluated, the statistical model included only the fixed effects of study diet, parity number, and days since calving, and the random residual term was used to determine whether body weight change differed for study diet groups. Additionally, sample status (standard sample or sample with intentionally added purified transgenic proteins), the interaction of study diet and sample status (fixed factors), and collection date were added as fixed independent variables to models for levels of CryIA(b) and PAT proteins in milk. Sample status and its interaction with study diet were tested using the residual term. These models allowed us to verify that 1) levels of CryIA(b) and PAT proteins detected in standard milk samples and samples with intentionally added transgenic proteins differed (indicated by a significant effect for sample status), 2) levels of transgenic proteins detected in milk samples from different study diet groups were similar (indicated by a non significant effect for study diet), and 3) any differences detected for levels of CryIA(b) and PAT proteins in milk for diet-sample status combinations were due to sample status and were independent of study diet consumed (indicated by a significant effect for sample status, a non significant effect for study diet, and a non significant effect for the interaction of these two effects).

Results and Discussion

Analyses of Diets for CryIA(b) and PAT Proteins   Results of the analyses of diets for CryIA(b) and PAT proteins are in Table 5. Diet samples were evaluated for 18, 19, 23, 24 September and 1 October 1996. In addition, analyses of samples for 23 September were repeated.

No CryIA(b) protein was detected in samples of diet that were prepared using fresh chopped whole-plant corn from the non-transgenic variety. Diets prepared using fresh whole-plant corn from the two transgenic varieties contained CryIA(b); levels of this protein were highest in diets prepared using the Bt11 derived variety. These results are consistent with patterns of CryIA(b) expression for Event 176 and Bt11 derived plants; Event 176 plants produce measurable amounts of CryIA(b) protein in green tissue and pollen only (Koziel et al., 1993; Fearing et al., 1997), and Bt11 plants produce measurable amounts of this transgenic protein in most plant tissues. Consequently, it was expected that diets prepared using the Bt11 variety would have the highest levels of CryIA(b) protein, and those prepared using the non-transgenic corn plants would contain no CryIA(b) protein.

For all diets that contained chopped whole-plant corn from transgenic corn hybrids, the lowest levels of CryIA(b) protein were for samples collected prior to day 5 of the study (22 September 1996). Days 1 through 4 constituted the acclimation period for this study, and during this period, the proportion of forages in the regular herd diet that was replaced by the respective chopped fresh whole-plant corn was increased incrementally. Consequently, the highest levels of transgenic proteins were anticipated for samples of diet that were collected after the acclimation period.

Analyses of Milk Samples for CryIA(b) and PAT Proteins   Results of qualitative and quantitative analyses for CryIA(b) and PAT proteins in milk produced by cows that consumed the three study diets are in Table 6.

Throughout the 14 days that milk samples were collected, no CryIA(b) or PAT protein was detected in any of the standard samples of milk that were collected from cows consuming the study diets. The Seeds Biotechnology Research Unit of Novartis Seeds, Research Triangle Park, NC, USA was unaware of identification for milk samples to which purified CryIA(b) and PAT proteins had been added intentionally, and this laboratory identified correctly all 55 samples that contained the added proteins (Table 6). For these samples, mean levels detected were similar for all study diet groups; mean levels of CryIA(b) protein were 1453, 1494, and 1399 ng/ml and levels of PAT protein were 567, 453, and 492 ng/ml for samples to which purified proteins had been added for the control corn, Event 176, and Bt11 study diets, respectively (Table 6). On average, levels detected represented 80% and 100%, respectively, of the CryIA(b) and PAT proteins that were added intentionally to selected milk samples. These results illustrate that, although CryIA(b) protein was present at detectable levels in diets fed to cows in the Event 176 and Bt11 study groups (Table 5), transgenic proteins were not present in milk produced by cows that consumed diets containing fresh chopped whole-plant *Bt* corn (Table 6).

Performance of Cows for Milk Yield, Feed Intake, and Milk Components

The daily intake of feed, production of milk, and levels in milk of fat, protein, lactose, solids-not-fat, other solids, and total solids for cows that consumed the study diets are represented as Least Squares Means in Table 7. For all study diet groups, means for milk yield include no data for 21, 22, and 25 September 1996, because these data were unavailable as a result of a problem with the computer system that was used by the dairy farm to collect milk yield data. Also, results for the Event 176 study diet group include data for 4 cows during days 1 to 7 and for 3 cows during days 8 to 14; cow #1198 was removed from the study on 26 September as a result of a digestive disorder. Details of the digestive disorder for cow #1198 are provided in a subsequent section of this report, "General Health and Clinical Observations."

Production of milk and intake of feed for cows in this study were high, and were typical for high producing Holstein dairy cows in the US (Table 7). On average, groups of cows that were fed study diets consumed more than 43 kg of feed (as fed) daily per cow and produced more than 38 kg of milk daily per cow. Composition of the milk was representative of composition for milk produced on commercial dairy farms in the US. Also, results in Table 7 indicate that when cows were fed diets prepared using chopped fresh whole-plant corn from the control, Event 176, and Bt11 varieties, milk yield, feed intake, and composition of milk were similar for all study diet groups. Statistical importance for factors other than study diet was examined, also.

Results for parity number, date sample was collected, and the number of days since calving are reported in Appendix 1; findings were not unusual.

General Health and Clinical Observations In general, cows remained healthy and in good body condition throughout the study. The nutrient demands are extremely great for high producing dairy cows that are in the early portion of lactation, and during this time period, cows typically lose body weight. Body weight changes (Appendix 2) for cows fed the control, Event 176, and Bt11 study diets were not different statistically ($P > 0.10$). Udder health was good for all cows, and no episodes of clinical mastitis occurred during the study. The latter finding was substantiated by counts of 339,000, 123,000, and 351,000 somatic cells per ml of milk from cows that consumed control, Event 176, and Bt11 study diets, respectively (Table 7). In addition, somatic cell counts for the three study diet groups were not different statistically ($P > 0.10$).

As noted previously, one cow (cow #1198) developed a rumen digestive disorder and was removed from the study on 26 September 1996. Dr. Karl Kersting was the attending clinician from the Iowa State University Veterinary Teaching Hospital who was responsible for veterinary care for cow #1198 following her removal from the study; a copy of Dr. Kersting's health report for this cow is included as Appendix 3.

Severe rumen digestive disorders can occur when cows are subjected to drastic and relatively abrupt changes in the forage component of the diet. To ensure that proteins associated with transgenic corn varieties were present in feed that was fed to cows during this study, considerable changes in the regular herd diet were needed. These changes included replacing ensiled alfalfa (lucerne silage) and corn silage with chopped fresh whole-plant corn from plants.

The requirement to harvest mature fresh corn plants at a stage of active photosynthesis limited the duration of the diet acclimation period. Levels of buffering agents in the diet (sodium bicarbonate and magnesium oxide) were increased to reduce the impact for cows of these abrupt diet changes, however, the necessary changes were sufficiently drastic and abrupt to induce severe rumen digestive upsets, such as the disorder that was experienced by cow #1198. The large daily variability of means for milk yield and feed intake for all study diet groups (Figures 1 and 2, respectively) likely indicates that all cows in the study were experiencing a subclinical level of rumen digestive upset that was initiated when cows were subjected to abrupt changes in the daily diet. However, no other cows required specialized care provided by a veterinarian, and, in fact, for all study diet groups, cows maintained a high level of milk yield during the 8 days preceding the study, 14 days of the study, and the 4 days following the end of the study (Table 8).

Summary and Conclusions

The primary objective for this study was to evaluate milk produced by dairy cows that were fed transgenic corn plants by determining whether milk produced by these cows contained CryIA(b) protein or its associated selectable marker protein, PAT. The individual corn varieties used for this study were selected because these are representative of commercial transgenic *Bt* and conventional hybrids that are marketed by Novartis Seeds in the US. In addition, selected varieties differed greatly for levels of transgenic proteins that they contained; the control variety contained no CryIA(b) protein, Event 176 derived corn plants contained intermediate levels, and plants from the Bt11 variety contained relatively high levels of CryIA(b) protein. Also, to provide ample opportunity for expression of transgenic proteins in milk, high producing cows that had high levels of feed intake were selected and fresh whole-plant corn from photosynthetically active plants constituted 100% of the forage for diets that were fed to cows. The Seeds Biotechnology Research Unit, Novartis Seeds documented subsequently that CryIA(b) protein was present in diets that contained whole-plant corn from Event 176 and Bt11 varieties, and that the CryIA(b) protein was not present in diets that contained corn plant material from the control variety.

The Seeds Biotechnology Research Unit of Novartis Seeds, Research Triangle Park, NC, USA detected no transgenic proteins in standard samples of milk collected from all cows, and identified correctly all 55 samples that contained intentionally added CryIA(b) and PAT proteins. Thus, if present in milk, the CryIA(b) insecticidal and PAT selective marker proteins can be detected. However, these proteins associated with transgenic corn hybrids were not detected in milk produced by cows that consumed diets containing substantial amounts (>30% of the diet as fed) of either non transgenic or Event 176 or Bt11 transgenic fresh chopped whole-plant corn. These findings were expected because it was anticipated that these transgenic proteins would be digested as conventional dietary proteins. Results from *in vitro* digestibility studies that used simulated mammalian gastric systems demonstrated that CryIA(b) is degraded immediately by gastric fluid (US Environmental Protection Agency, 1997a; 1997b; Novartis Seeds unpublished data).

Generally, health for cows fed study diets was good as evidenced by no clinical udder health problems, and by high levels of milk yield prior to, during, and following the end of the study period. One cow developed a digestive disorder and was removed from the study, however, there is no evidence to suggest a relationship between the digestive disorder and the presence in the diet of transgenic corn. Instead, it is likely that the rumen digestive upset experienced by this cow was initiated when cows were subjected to abrupt and drastic changes in the forage portion of the daily diet.

Our findings indicate clearly that the CryIA(b) and PAT proteins cannot be detected in milk from cows that are consuming diets prepared using transgenic corn varieties that produce these proteins. Moreover, we detected no adverse effects of these transgenic proteins or unintended effects on forage nutritional quality for dairy cows that consumed green plant material from Novartis Seeds' transgenic *Bt* corn hybrids.

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Table 1. Level of CryIA(b) insecticidal protein detected in chopped corn plant material and prepared diet for 18 September 1996.

Corn Variety	*Fresh whole chopped corn plants*	*Prepared diet*
	—— CryIA(b), ng/g fresh weight ——	
Control	66.4[1]	0.0
Event 176	87.6	10.9
Bt11	635.7	27.1

[1]Value of 0.0 was expected, and was confirmed by 0.0 ng CryIA(b) protein per g of corresponding prepared diet sample. Inadvertent contamination of fresh chopped corn sample likely occurred prior to laboratory analysis.

Table 2. Averages when assigned to study diet groups for several characteristics of cows.[1]

Characteristic	*Study diet consumed*			*Largest*
	Control	*Event 176*	*Bt11*	*SEM[2]*
Body weight, kg	636	578	605	22.4
Days since calving	38.3	89.5	72.3	33.1
Energy corrected milk, kg/day	39.4	40.6	42.5	4.28
Milk, kg/day	37.5	40.3	41.7	4.13
Milk fat, %	4.08	3.68	3.72	.505
Milk protein, %	2.85	2.78	2.92	.194

[1]Averages based on 4 cows per group.
[2]Largest standard error of the mean for diet study groups.

Table 3. Results of proximate analyses for chopped whole-plant corn collected on 19 September 1996 from one non-transgenic and two transgenic *Bt* varieties that were used in study diets.[1]

Analyte	*Control*	*Event 176*	*Bt11*
		%	
Total Moisture	58.8	61.6	71.0
Protein	2.55	3.04	2.00
Total Digestible Nutrients	29.3	27.3	19.3
Neutral detergent fiber	18.0	16.2	14.8
Acid detergent fiber	9.8	9.2	8.9
Minerals			
Calcium	0.08	0.09	0.08
Magnesium	0.11	0.08	0.06
Phosphorus	0.08	0.09	0.06
Potassium	0.28	0.28	0.39
Sulfur	0.04	0.04	0.03
Sodium	0.010	0.010	0.010
		ppm	
Zinc	8	9	5
Manganese	11	8	5
Copper	2	3	2
Iron	78	70	37
Cobalt	<1.0	<1.0	<1.0
Aluminum	44	33	10
Energy		*kcal/kg*	
Metabolizable energy	1060	988	699
Net energy lactation	681	633	423
Net energy growth	414	384	251
Net energy maintenance	666	619	432

[1]Iowa Testing Laboratories, Inc., Eagle Grove, Iowa, USA

Table 4. Composition (as fed) of study diets.

	Corn variety used in diet		
Ingredient[1]	***Control***	***Event 176***	***Bt11***
		% as fed	
Whole-plant chopped corn[2]	30.8	33.8	37.4
Wet corn gluten feed	26.4	25.0	23.9
Corn grain	11.6	11.0	10.5
Whole cottonseed	6.3	6.0	5.7
Roasted soybeans	3.7	3.5	3.3
Minerals	2.57	2.43	2.32
Alfalfa hay	18.6	17.6	16.8
Total	100.0	100.0	100.0

[1]Except for whole-plant chopped corn, all feed ingredients were from standard commercial feed suppliers.

[2]Quantities of whole-plant chopped corn (as fed) were adjusted when significant change in moisture content was detected.

Table 5. Level of CryIA(b) protein in diets prepared using chopped whole-plant corn from one non-transgenic and two transgenic *Bt* varieties.[1]

	Corn variety used in diet		
Date collected	***Control***	***Event 176***	***Bt11***
	— ***CryIA(b), ng/g fresh weight*** —		
Acclimation Period			
18 September 1996	0	10.9	27.1
19 September 1996	0	13.5	76.2
Main Study Period			
23 September 1996	0	21.1	629.1
second sample	0	31.3	332.6
third sample	0	36.0	494.9
24 September 1996	0	48.4	306.4
1 October 1996	0	28.6	357.1

[1]Seeds Biotechnology Research Unit, Novartis Seeds, Research Triangle Park, NC, USA.

Table 6. Means for level of CryIA(b) and PAT proteins in milk samples from six sources.

Source of sample	*No. samples*	*CryIA(b) protein*	*PAT protein*
		ng/ml	
Standard sample[1]			
Study diet consumed			
Control	80	0	0
Event 176	67	0	0
Bt11	80	0	0
Largest SEM[3]		0.0	0.0
Purified CryIA(b) and PAT proteins added intentionally[1, 2]			
Study diet consumed			
Control	20	1453	567
Event 176	17	1494	453
Bt11	18	1399	492
Largest SEM[3]		91.9	103.1

[1]For levels of CryIA(b) and PAT proteins in milk, sample sources differed ($P < 0.0001$), but effects of diet and diet × sample source were statistically unimportant.

[2]Concentrations in milk samples of intentionally added purified CryIA(b) and PAT proteins were 1.8 and 0.5 μg/ml, respectively

[3]Largest standard error of the mean for respective sample sources from diet study groups.

Table 7. Least Squares Means of milk yield, somatic cell count, and milk components collected during 18 September to 1 October 1996 for cows that consumed three study diets.

	Study Diet Consumed			*Largest*
Performance Measure	***Control***	***Event 176[1]***	***Bt11***	***SEM[2]***
Milk, kg/day[3,4,5]	40.4	39.5	38.2	1.96
Feed Intake, kg as fed/day[4,5]	43.4	44.8	47.0	.999
Somatic Cell Count, cells $\times$ 10^{-3}/ml[4,5]	339	123	351	234
Fat, %[4,5]	3.41	3.50	3.47	.183
Protein, %[4,5]	2.72	2.66	2.80	.082
Lactose, %[4,5]	4.77	4.78	4.88	.067
Solids-not-fat, %[4,5]	11.59	11.63	11.84	.289
Other Solids, %[4,5]	8.18	8.12	8.37	.117
Total Solids, %[4,5]	16.9	17.2	19.4	1.38

[1]Includes data for 4 cows (days 1-7) and 3 cows (days 8-14).

[2]Largest standard error of the mean for diet study groups.

[3]Milk yield data were not available for 21, 22, and 25 September 1996 (days 3,4, and 7) as a result of problems with the farm computer system.

[4]Linear contrast of difference between means for Event 176 and Bt11 was not important statistically ($P > 0.10$).

[5]Linear contrast of difference between means for Control corn and the average of Event 176 and Bt11 was not important statistically ($P > 0.10$).

Table 8. Least Squares Means of daily milk yield prior to, during, and following the study period for three diet study groups.

Period	*Study Diet Consumed* Control	Event 176	Bt11	*Largest SEM*[1]
	kg			
8 days prior to study	39.8	38.6	37.3	1.26
14 day study period[2]	40.4	39.5[3]	38.2	1.96
4 days following study	35.9	39.7[4]	39.0	2.48

[1]Largest standard error of the mean for diet study groups.
[2]Milk yield data were not available for 21, 22, and 25 September 1996 (days 3,4, and 7) as a result of problems with the farm computer system.
[3]Includes data for 4 cows (days 1, 2, 5, 6) and 3 cows (days 8-14).
[4]Includes data for 3 cows.

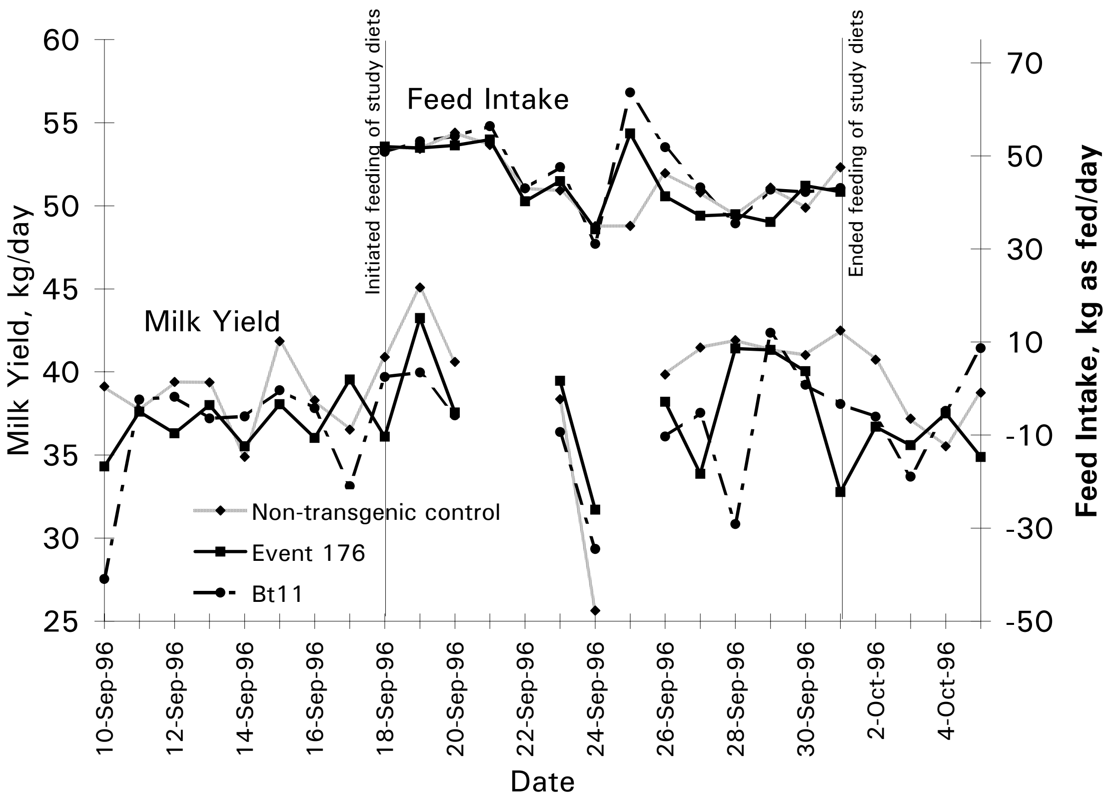

Figure 2. Milk yield and feed intake for groups fed study diets.

Appendix 1. Importance of parity number, date sample was collected, and days since calving for several measures.

	Source of Variation		
***Measure*[1]**	***Parity number***	***Date sample collected***	***Days since calving***
		P-value	
Daily Milk Yield	> 0.10	< 0.0001	0.068
Daily Feed Intake, as fed	> 0.10	< 0.0001	0.084
Somatic Cell Count	> 0.10	> 0.10	> 0.10
Fat %	> 0.10	< 0.0001	> 0.10
Protein %	> 0.10	0.027	> 0.10
Lactose %	0.100	0.001	0.002
Solids-Not-Fat %	> 0.10	< 0.0001	> 0.10
Other Solids %	0.085	0.005	0.095
Total Solids %	> 0.10	< 0.0001	> 0.10
CryIA(b) Protein	> 0.10	0.003	> 0.10
PAT Protein	> 0.10	< 0.0001	> 0.10
Body Weight Change	> 0.10	—	> 0.10

[1]Statistical models for measures are described in the section, "Statistical Analyses".

Appendix 2. Characteristics for cows fed study diets.

Cow	*Study diet*	*Parity no.*	*Days since calving*[1]	*Energy Corrected Milk*[1]	*Body weight 18 Sept.*[2]	*Body weight 2 Oct.*[3]
				kg	*kg*	*kg*
1016	Control	4	66	40.7	696	703
1046	Bt11	3	185	50.0	673	645
1112	Event 176	3	68	49.8	626	623
1158	Event 176	2	162	40.1	560	573
1178	Bt11	2	61	39.8	558	547
1198	Event 176	2	54	43.1	542	---[4]
1210	Control	2	82	44.9	620	607
1221	Bt11	2	62	42.4	601	608
1225	Bt11	2	93	37.5	583	580
1226	Control	2	44	43.3	590	657
9854	Control	5	73	28.2	630	637
9992	Event 176	4	186	29.2	578	562

[1]As reported when cows were assigned to study diet groups.
[2]Weight collected at the start of the trial.
[3]Weight collected at the end of the trial.
[4]No weight collected, because cow was removed from the study on 26 September 1996.

Appendix 3. Veterinarian's Report

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Department of Veterinary Clinical Sciences
Veterinary Teaching Hospital
Ames, Iowa 50011-1250
Large Animal 515 294-1500
Small Animal 515 294-4900
Field Service/Production Medicine 515 294-5006
FAX 515 294-9281

October 17, 1996

Dr. Marjorie Faust
Extension Dairy Science
4 Kildee Hall
Ames, IA 50011-3150

RE: Case #267545

Dear Marj:

This is a final report on cow #1198 presented to the Iowa State University Veterinary Teaching Hospital on September 26, 1996 from the ISU Dairy Farm (Ames). Clinical and laboratory findings were consistent with a diagnosis of acute engorgement and rumen acidosis with severe secondary electrolyte disorders. The animal became a "downer" despite therapy and was dispatched on October 4, 1996. Post-mortem examination of the rumen revealed considerable mucosal sloughing. The final diagnosis was toxic indigestion/rumen acidosis secondary to green chop corn engorgement.

Marj, please let me know if you have further questions.

Sincerely,

Karl

Karl Kersting, DVM, MS
Section Head
Food Animal Medicine and Surgery

KK/jc

Application for renewal of authorization of Bt11 maize and derived products notified according to Articles 11 and 23 of Regulation (EC) No 1829/2003 on genetically modified food and feed

PART I: TECHNICAL DOSSIER

APPENDIX 8.3

Evaluation of transgenic Bt11 hybrid corn in broiler chickens

Evaluation of Transgenic Event Bt11 Hybrid Corn in Broiler Chickens[1]

J. Brake,*[,2] M. A. Faust,† and J. Stein‡

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ABSTRACT A feeding study evaluated whether standard broiler diets prepared with grain derived from Syngenta Seeds NK Brand *Bacillus thuringiensis* (Bt) Corn hybrids had any adverse effects on male or female broiler chickens. Four kinds of corn grain were used in this study: (1) grain from the Bt-expressing field corn hybrid N7070Bt, (2) grain from the N7070Bt hybrid that had been sprayed with Liberty brand herbicide (glufosinate) according to manufacturer's instructions (N7070Bt + Liberty), (3) grain from standard N7070 (non-Bt isoline of N7070Bt) grain, and (4) a lot of North Carolina grown grain from the 2000 growing season (NC2000).

The amino acid balance for the four lots of corn was similar relative to their crude protein content; however, the NC2000 corn had higher protein content. Diets with the higher protein NC2000 season corn were amended with a combination of sand, ground cardboard (Solka Floc), and poultry fat so that the metabolizable energy and crude protein content of the diluted diets would be similar to that of the isoline and transgenic diets.

Growth of broilers was excellent with males being significantly heavier than females (2,497 g vs. 2,103 g) at 42 d of age. BW of live birds at 42 d was within 26 g for the three treatment groups fed corn that was from the same genetic background, i.e., the two Bt transgenic groups (N7070Bt, N7070Bt + Liberty), and the non-Bt N7070 isoline corn group, while BW for the NC2000 group was significantly lower by 93 g. There was no overall corn source effect on feed conversion ratio (FCR) among the isoline and transgenic corn sources to 42 d of age, but FCR was poorer for broilers consuming the commercial NC2000 corn. There was no overall effect of corn source on survivability to 42 d. Carcass analysis at 48 d demonstrated no differences in percentage carcass yield due to corn source among males and females.

The transgenic N7070Bt and N7070Bt + Liberty hybrid diets supported excellent broiler chicken growth with mortality and FCR that were similar to that supported by the N7070 isoline control and better than rates from the commercial NC2000 corn without significant differences among treatment groups in carcass yield. It was clear that the transgenic corn had no deleterious or unintended effects on production traits of broiler chickens in this study.

(*Key words*: transgenic corn, transgenic maize, N7070Bt corn, N7070Bt maize, broiler)

2003 Poultry Science 82:551–559

INTRODUCTION

Agricultural biotechnology has produced a number of new varieties of crop plants with enhanced features, such as protection against common pests, tolerance to herbicides, and improved quality traits. The first commercial plantings of insect-protected field corn hybrids that utilized the "Bt" genetic modification, designated "Event 176," occurred in 1996. These corn hybrids (including those derived from Event Bt11, the subject of this study) express an insecticidal protein, Cry1Ab, similar to that produced in nature by certain subspecies of the common soil bacterium *Bacillus thuringiensis* (Bt) (Koziel et al., 1993). The Cry1Ab protein is selectively toxic to the larvae of the European Corn Borer and certain other lepidopteran larvae while nontoxic to other orders of insects as well as animals and humans. Commercial formulations of Bt have been used as topical insecticides since 1938, and such Bt-based products have been registered for use on food crops in the U.S. since 1961 [U.S. Environmental Protection Agency (EPA), 1986]. Currently, there are eight different Bt events in corn that have successfully completed the U.S. Food and Drug Administration (FDA), U.S. Department of Agriculture, and EPA review processes and are available commercially (U.S. FDA, 2002).

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[1]The use of trade names in this publication does not imply endorsement of the products mentioned nor criticism of similar products not mentioned.

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Abbreviation Key: Bt = *Bacillus thuringiensis*; FCR = feed conversion ratio; Liberty = glufosinate herbicide; N7070 = Northrup King brand corn hybrid N7070; NC2000 = North Carolina grown corn from 2000 growing season.

In addition to expressing the Cry1Ab insecticidal protein, Event Bt11 field corn also expresses a second novel protein, phosphinothricin acetyltransferase. This protein inactivates phosphinothricin, the active component in Liberty herbicide. Liberty is a postemergence herbicide registered for use on corn. Unlike most weeds present in corn fields, corn plants expressing the phosphinothricin acetyltransferase protein do not suffer damage when sprayed with Liberty herbicide when it is applied according to manufacturer's instructions.

The purpose of this study was to evaluate whether feed comprised of transgenic grain derived from Syngenta Seeds Event Bt11 (N7070Bt) hybrid field corn or N7070Bt hybrid corn that had been sprayed with Liberty herbicide (N7070Bt + Liberty) had any adverse effects on broiler chickens when compared with feed comprised of grain derived from a nontransgenic isoline. In the U.S., chickens (*Gallus domesticus*) consume large quantities of corn grain in commercial feeds. Broiler chickens, in particular, have relatively high corn consumption because conventional feeding regimens are designed to provide maximal BW gain in the shortest time. Although the transgenic protein is present in corn grain in parts per billion quantities, it was of interest to determine whether the transgenic corn grain had any unexpected effects on poultry, either as a direct effect of transgenic protein in the diet or as a result of any unintended compositional changes in the grain that may have altered its nutritional value. In order to determine whether transgenic-derived corn has an effect on broiler chicken performance, this study compared male and female broiler chickens receiving feed made with corn from a transgenic hybrid (N7070Bt and N7070Bt + Liberty) vs. feed made with the corresponding nontransgenic (N7070 isoline) version of the same hybrid and a locally (North Carolina) grown commercial lot of corn from the 2000 growing season (NC2000). A similar model has been used to study Bt corn (Halle et al., 1998; Leeson, 1998; Brake and Vlachos, 1999; Mirales et al., 2000; Gaines et al., 2001; Piva et al., 2001; Taylor et al., 2001a,b) and glyphosphate-tolerant corn (Sidhu et al., 2000; Gaines et al., 2001; Taylor et al., 2001a,b) as feeds for broiler chickens. The performance endpoints measured in this study included effects on survival, BW, feed efficiency, and carcass yield.

MATERIALS AND METHODS

Test and Control Corn

The transgenic corn grain used in this study was from both Syngenta Seeds' Event Bt11 hybrid N7070Bt and the same Bt hybrid that had been sprayed according to manufacturer's instructions with a single application of Liberty herbicide (N7070Bt + Liberty) according to manufacturer's instructions. The hybrids were field-grown in Hawaii in 2001. Following grain harvest, shelling and storage were performed using standard procedures and conditions. The presence of Cry1Ab protein in N7070Bt grain was confirmed by ELISA, and the protein was present in the grain at the expected level (~800 ng/g seed). The nontransgenic control corn grain used in this study was from Syngenta Seeds' N7070 hybrid, an isogenic (isoline) conventional hybrid, derived from the same inbred parents as the transgenic lines that was grown, processed, and stored concurrently with the transgenic hybrid under the same environmental conditions. Isolation procedures ensured that intermixing of grain genotypes did not occur. In addition, a locally grown lot of North Carolina corn from the 2000 season (NC2000) was used for comparison purposes. The NC2000 season corn was in storage at the time of the experiment and was known to be of adequate quality to support good broiler chicken growth.

Experimental Design

The experimental design consisted of diets made from four types of corn (transgenic N7070Bt and N7070Bt + Liberty, nontransgenic isoline N7070, and locally grown NC2000 corn) fed to two sexes of birds housed within four location blocks of the growing facility with eight replicates for each two-way corn source × sex interaction. Pens were assigned in a randomized complete block design to compensate for known location effects in the growing facility.

Broiler chicks were hatched from commercial hatching eggs incubated at the site. The broiler breeder parent stock was a commercial strain of Ross males and feather-sexable females. Broiler chicks were feather-sexed and very large and small chicks were excluded. Furthermore, any chicks exhibiting obvious abnormalities were not used in the study. A total of 1,600 birds were randomly distributed into 64 pens of a curtain-sided house at 1 d of age so that pens contained 25 birds of the same sex. Birds were identified by neck tag indicating animal number.

Corn Analyses

Samples of each of the four lots of corn were forwarded to independent laboratories using standard methods (AOAC, 1990, 1993) for proximate analyses, amino acid analyses, and mycotoxin screening. Results are shown in Table 1 and are reported as the percentage by weight on an as-is basis (i.e., not as a percent of the dry weight). Amino acid analyses showed very similar amino acid patterns for samples of transgenic (N7070Bt and N7070Bt + Liberty), isoline (N7070), and NC2000 corn and confirmed differences in crude protein and moisture between the N7070-based lots and the NC2000 corn. Initial routine mycotoxin determinations showed extremely low contamination for both aflatoxins and deoxynivalenol (vomitoxin) (Table 1) in all corn samples. There was some evidence of fumonisin in all of the corn samples. However, the levels of mycotoxins found in the corn were not excessive by commercially accepted standards. By comparison, feeding of diets containing deoxynivalenol at levels approaching 500 ppb is frequent in commercial broiler practice without obvious effect.

TABLE 1. Analyses of corn samples[1]

Analyses[2]	Corn source			
	N7070 Isoline	N7070Bt	N7070Bt + Liberty	NC2000
Proximate analyses,[3] %				
Moisture	10.52	10.94	10.73	12.76
Fat	3.39	3.21	3.03	3.53
Protein	7.79	7.60	7.71	8.69
Fiber	1.30	1.20	1.30	1.93
Ash	1.27	1.18	1.27	1.22
Amino acids,[4] %				
Taurine	0.15	0.19	0.18	0.17
Hydroxyproline	0.02	0.02	0.02	0.02
Aspartic acid	0.53	0.54	0.58	0.56
Threonine	0.26	0.26	0.27	0.29
Serine	0.31	0.30	0.33	0.35
Glutamic acid	1.46	1.44	1.57	1.73
Proline	0.70	0.68	0.73	0.81
Lanthionine	0.00	0.00	0.00	0.00
Glycine	0.30	0.30	0.31	0.35
Alanine	0.57	0.57	0.62	0.67
Cysteine	0.19	0.18	0.20	0.21
Valine	0.38	0.38	0.39	0.45
Methionine	0.15	0.15	0.16	0.19
Isoleucine	0.26	0.25	0.27	0.31
Leucine	0.90	0.86	0.93	1.11
Tyrosine	0.23	0.22	0.24	0.26
Phenylalanine	0.37	0.36	0.39	0.45
Hydroxylysine	0.00	0.00	0.00	0.00
Histidine	0.22	0.22	0.24	0.27
Ornithine	0.01	0.01	0.01	0.01
Lysine	0.26	0.25	0.27	0.27
Arginine	0.37	0.36	0.38	0.44
Tryptophan	0.05	0.06	0.06	0.07
Total	7.69	7.60	8.15	8.99
Mycotoxin analyses[5]				
Aflatoxins, ppb	ND[6]	ND	ND	ND
Deoxynivalenol, ppb	ND	ND	ND	ND
Fumonisin B_1, ppm	20.6	9.9	15.6	8.8

[1]N7070 = Northrup King brand corn hybrid N7070; Bt = *Bacillus thuringiensis*; Liberty = glufosinate herbicide; NC2000 = North Carolina grown corn from 2000 growing season.

[2]Reported on an as-is basis.

[3]Woodson-Tenent Laboratories, Goldston, NC.

[4]Experiment Station Chemical Laboratory, Univ. of Missouri, Columbia, MO.

[5]Trilogy Analytical Laboratory, Inc., Washington, MO.

[6]Not detected.

Diet Formulation

Based on the initial proximate analyses (Table 1), grain from the three Syngenta Seed's N7070 series hybrids were assumed to have the same nutritive value. To compensate for the relatively large difference in percentage crude protein between the NC2000 and N7070-based corn sources, an inert nonnutritive filler composed of Solka Floc 40 (cardboard) and sand, formulated to the same density as ground corn, was added to volume. Furthermore, an appropriate quantity of additional poultry fat was added as a portion of the filler to compensate for the ME lost due to removal of a portion of the corn and replacement with the nonnutritive filler. Diet formulation was on the basis of the N7070 isoline corn with filler added as described above for the respective diets made from the NC2000 corn (Table 2). The N7070 basal diet formulation reflected commercial practice at the time of the study. Nutrient levels met or exceeded the minimum nutritional requirements for broiler chickens as set by the National Research Council (1994). Diets were pelleted in a small commercial-style pellet mill immediately after mixing. Pelleted starter feed was crumbled.

Access to Feed and Water

Birds were provided continuous access to feed and water for ad libitum consumption from two tube feeders and one automatic bell-type waterer in each pen. Supplemental waterers as well as supplemental feeder flats were used during the first week to ensure unlimited access to feed and water. The feeders were manually agitated as needed to maintain the flow of feed from the tube into the feeder pan from which the birds fed. Birds had access to 1.13 kg starter diet per bird during the first 21 d of the study. This was followed by the grower diet, which was

TABLE 2. Diets and calculated analyses by corn source[1]

	Starter diets				Grower diets				Finisher diets			
Ingredient	N7070 Isoline	N7070Bt	N7070Bt + Liberty	NC2000	N7070 Isoline	N7070Bt	N7070Bt + Liberty	NC2000	N7070 Isoline	N7070Bt	N7070Bt + Liberty	NC2000
	(% by weight)											
Corn	48.19	48.19	48.19	47.79	56.88	56.88	56.88	51.54	63.56	63.56	63.56	57.56
Soybean meal	41.05	41.05	41.05	41.07	33.64	33.64	33.64	33.65	28.33	28.33	28.33	28.33
Limestone (calcium carbonate)	1.10	1.10	1.10	1.09	1.12	1.12	1.12	1.13	1.02	1.02	1.02	1.02
Dicalcium phosphate	1.72	1.72	1.72	1.71	1.77	1.77	1.77	1.77	1.53	1.53	1.53	1.53
Poultry fat	6.54	6.54	6.54	6.65	5.35	5.35	5.35	7.37	4.22	4.22	4.22	6.51
DL-Methionine	0.24	0.24	0.24	0.25	0.08	0.08	0.08	0.08	0.03	0.03	0.03	0.03
Trace mineral premix	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt (NaCl)	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.33	0.33	0.33	0.33
Coccidiostat (Monensin)	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Selenium premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sand filler	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.82	0.03	0.03	0.03	0.95
Solka Floc filler[2]	0.00	0.00	0.00	0.18	0.00	0.00	0.00	2.48	0.04	0.04	0.04	2.83
Sodium bicarbonate	—	—	—	—	—	—	—	—	0.18	0.18	0.18	0.18
Ascorbic acid	—	—	—	—	—	—	—	—	0.11	0.11	0.11	0.11
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analyses[3]												
Metabolizable energy, kcal/g	3.20	—	—	—	3.20	—	—	—	3.20	—	—	—
Crude protein, %	23.47	—	—	—	20.50	—	—	—	18.50	—	—	—
Lysine, %	1.35	—	—	—	1.15	—	—	—	1.00	—	—	—
Methionine + cystine, %	0.99	—	—	—	0.75	—	—	—	0.65	—	—	—
Threonine, %	0.90	—	—	—	0.78	—	—	—	0.71	—	—	—

[1]N7070 = Northrup King brand corn hybrid N7070; Bt = *Bacillus thuringiensis*; Liberty = glufosinate herbicide; NC2000 = North Carolina grown corn from 2000 growing season.

[2]Fibre Sales and Development Corporation, Checkerboard Square, St. Louis, MO.

[3]Based on analytical values used for formulation and National Research Council (1994) reference values. All formulas were derived from the base formulation with N7070. The NC2000 diet was developed by reduction of crude protein by addition of a sand and Solka Floc filler that had the same density as corn and poultry fat to compensate for metabolizable energy lost with the corn that was removed.

added to any starter diet that remained in the feeders at 21 d. At 35 d, remaining grower diet was weighed and discarded and a finisher diet was added to the feeders. Feed was available for ad libitum consumption except at 42 and 43 d of the experiment when access to feed was limited to control heat-stress-related mortality. Also, access to feed was discontinued approximately 12 h before slaughter on the night of the 47th d of the experiment.

Housing and Environmental Conditions

Birds were housed in 1.2- × 3.7-m pens with pine shavings as litter. House temperature was 32°C for the first week of the experiment and decreased gradually until ambient summer conditions were reached. Temperature was maintained using thermostatically controlled liquid propane (LP) gas brooders and circulating fans. The house was ventilated using stirring fans and manually operated curtains. The birds received 23 h of incandescent light per day until 7 d of age, 21 h of light from 8 d to 28 d of age, 20 h of light from 29 to 35 d of age, and 19 h of light for the remainder of the experiment.

Heat Stress Incident

At 41 d of the experiment, there was a sudden and extreme increase in ambient temperature and humidity. The heat index exceeded 43°C during the afternoon, and birds began to die. All mortalities were weighed as quickly as possible, and on the morning of 42 d, the decision was made to terminate the growth performance portion of the experiment by weighing all live birds and total feed consumed. For completeness, BW of live and dead birds are presented; however, group differences were minor and unimportant. Access to feed was limited on 42 and 43 d of the experiment to allow broilers to lose body heat. After the ambient heat stress subsided, the broilers were returned to full feed for 44 d through 47 d of the experiment prior to processing.

Data Collection

Total pen weight data were collected at hatch (1 d), 21 d, 35 d, and 42 d of age. All birds that died were weighed as soon as possible after death. At the latter three time points, feed consumption per pen was determined for calculating the adjusted FCR.[3] At 48 d after recovery from the heat stress, a random sample of two birds from each pen was processed in order to determine carcass (meat) yield. These birds were stunned, killed by exsanguination, scalded, picked, eviscerated, and deboned as previously reported (Brake et al., 1993).

Statistical Analyses

The data for BW, FCR, and survival were analyzed to determine statistical differences for corn diets and sex. Statistical analyses were performed using the general linear model (GLM) procedure of SAS Institute (1999) with sex and corn source as independent variables in a two-way analysis of variance within a randomized complete block design, with random error (between-pen variation) as the error term. Individual broiler carcass data on gross and adjusted to BW (%) bases were analyzed for effects due to corn source within sex using a one-way analysis of variance, as carcass effects due to sex are well known. Statements of statistical significance were based upon $P < 0.05$.

RESULTS AND DISCUSSION

Diet Analyses

The percentages of crude protein, moisture, fat, fiber, and ash of the formulated diets, as determined by proximate analyses, are shown in Table 3. Variability was within the normally expected range. Percentage dietary fat varied somewhat, and the NC2000 diets appeared to contain slightly more fat overall as expected. This was probably a function of the additional poultry fat filler used to compensate for the ME lost from diluting the corn portion of the diets. These differences were not substantial but could have small effects on BW and FCR. The percentages of fiber and ash were obviously higher for the NC2000 corn only in the finisher diet. These observations reflect the higher inclusion rate of sand (ash) and Solka Floc (fiber) in the NC2000 finisher diet.

Body Weight Data

The effect of corn source and sex on BW is shown in Table 4. There were no significant interactions between corn source and sex at any age. The results showed that at hatch and placement (1 d) there were no differences in the BW of all chicks placed in the study. As expected, by 21 d of age, sex effects became evident with the males weighing significantly more than the females. These effects were also observed at 35 and 42 d. There were significant BW differences due to corn source at 21, 35, and 42 d of age for live birds; the birds fed the NC2000 diets exhibited the lowest BW throughout the experiment. This likely was due to an interaction between the climatic conditions and the diet formulation that affected feed intake (as discussed below) and, consequently, reduced growth rates for NC2000 groups. Birds fed the isoline N7070 and transgenic N7070Bt and N7070Bt + Liberty corn source diets performed in a statistically similar manner at all ages. For the 42 d measure, separate results for live and dead birds are presented to account for birds that died as a result of the heat stress at 41 and 42 d (Table 4). However, relatively little difference between diet groups in BW occurred for the live and dead birds.

[3]Adjusted feed conversion ratio (calculated for each pen) = total feed consumed/total BW of surviving birds + total terminal BW of birds that died.

TABLE 3. Analyses of formulated diet samples

Diet analyses[1]	N7070 Isoline	N7070Bt	N7070Bt + Liberty	NC2000
	Corn source[2]			
Percentage crude protein				
Starter	24.10	24.62	25.43	23.49
Grower	23.24	22.20	22.72	25.20
Finisher	17.92	19.44	18.97	20.32
Percentage moisture				
Starter	10.69	11.35	11.27	11.61
Grower	11.16	11.32	11.35	11.59
Finisher	10.99	11.49	11.05	11.39
Percentage fat				
Starter	8.35	7.87	7.90	8.06
Grower	7.24	7.43	6.80	7.64
Finisher	6.51	6.24	6.08	8.22
Percentage fiber				
Starter	2.10	2.40	2.00	1.90
Grower	1.90	2.10	2.00	2.00
Finisher	2.50	2.60	2.40	2.80
Percentage ash				
Starter	5.72	5.86	5.56	5.59
Grower	5.73	5.18	5.61	5.62
Finisher	4.67	4.69	4.86	5.21

[1]Analyses performed by Woodson-Tenent Laboratories, Goldston, NC, on an as-is basis.

[2]N7070 = Northrup King brand corn hybrid N7070; Bt = *Bacillus thuringiensis*; Liberty = glufosinate herbicide; NC2000 = North Carolina grown corn from 2000 growing season.

There were no sex × corn source interactions; males typically grew faster than females. Piva et al. (2001) reported that birds fed diets containing Bt corn were slightly heavier at finish than birds fed the non-Bt isoline control hybrid, while results from other studies indicated no impact of Bt hybrids for final BW and BW gain in broilers (Halle et al., 1998; Brake and Vlachos, 1999; Mirales et al., 2000; Taylor et al., 2001a,b).

Feed Conversion

The effect of corn source and sex on FCR during individual feeding periods and cumulatively are shown in Table 5. There were no significant interactions between corn source and sex at any age. It is well established that males exhibit better FCR than do females, as found in this study (Table 5). Corn source had a significant effect on FCR during the starter period (0 to 21 d), finisher period (35 to 42 d), and cumulatively (0 to 42 d) due to poorer performance from broilers fed the NC2000 diets. Groups fed the isoline N7070 and transgenic N7070Bt and N7070Bt + Liberty corns performed in a statistically similar manner. There was no interaction of sex × corn source for FCR.

Although efforts were made in this study to adjust diets for observed small differences in corn source (NC2000

TABLE 4. The effect of corn source and sex on BW of broiler chickens[1,2]

Corn source[3]	Sex	BW at hatch Mean	BW at hatch SEM	BW at 21 d Mean	BW at 21 d SEM	BW at 35 d Mean	BW at 35 d SEM	BW at 42 d (live) Mean	BW at 42 d (live) SEM	BW at 42 d (dead) Mean	BW at 42 d (dead) SEM	BW at 42 d (live + dead) Mean	BW at 42 d (live + dead) SEM
		(g)											
N7070 Isoline		37.8	0.18	824.4[a]	10.81	1,841.8[a]	35.13	2,329.0[a]	52.60	2,246.9	97.59	2,319.6[a]	51.19
N7070Bt		37.7	0.22	809.6[a]	12.58	1,816.7[ab]	36.68	2,303.7[a]	54.14	2,213.7	73.58	2,285.2[ab]	52.23
N7070Bt + Liberty		37.5	0.29	809.0[a]	9.91	1,820.6[ab]	35.09	2,319.7[a]	51.17	2,239.6	47.62	2,309.8[a]	50.26
NC 2000		38.0	0.24	773.5[b]	10.74	1,790.0[b]	38.53	2,210.5[b]	59.00	2,224.5	74.13	2,208.7[b]	58.76
P-value effect of corn source		0.50		0.0001		0.02		0.003		0.99		0.02	
	Male	37.9	0.17	841.2[a]	5.56	1,951.5[a]	9.02	2,496.5[a]	16.41	2,411.8[a]	32.02	2,479.9[a]	17.15
	Female	37.5	0.15	767.1[b]	4.62	1,683.1[b]	7.77	2,102.9[b]	11.25	2,046.1[b]	44.98	2,098.7[b]	11.11
P-value effect of sex		0.11		0.0001		0.0001		0.0001		0.0001		0.0001	

[a,b]Means with different superscripts differ significantly ($P \leq 0.05$).

[1]Starter, grower, and finisher diets were used to 21, 35, and 42 d of age, respectively.

[2]There were eight replicate pens of 25 birds each for each interaction mean except for the NC 2000 treatment after 35 d for which six replicate pens were used.

[3]N7070 = Northrup King brand corn hybrid N7070; Bt = *Bacillus thuringiensis*; Liberty = glufosinate herbicide; NC2000 = North Carolina grown corn from 2000 growing season.

TABLE 5. The effect of corn source and sex on adjusted feed conversion ratio (FCR) of broiler chickens[1,2]

Corn source[3]	Sex	Starter FCR 0–21 d Mean	SEM	Grower FCR 21–35 d Mean	SEM	Finisher FCR 35–42 d Mean	SEM	Cumulative FCR 0–35 d Mean	SEM	Cumulative FCR 0–42 d Mean	SEM
		(g:g)									
N7070 Isoline		1.32[b]	0.01	1.81	0.01	3.19[b]	0.14	1.60	0.01	1.92[b]	0.02
N7070Bt		1.33[b]	0.01	1.83	0.04	3.20[b]	0.13	1.61	0.02	1.94[b]	0.03
N7070Bt + Liberty		1.33[b]	0.00	1.77	0.03	3.26[b]	0.14	1.58	0.01	1.93[b]	0.03
NC 2000		1.37[a]	0.01	1.79	0.01	3.73[a]	0.24	1.61	0.01	2.01[a]	0.03
P-value effect of corn source		0.0002		0.30		0.02		0.26		0.005	
	Male	1.33[b]	0.01	1.76[b]	0.01	2.84[b]	0.06	1.58[b]	0.01	1.84[b]	0.01
	Female	1.35[a]	0.00	1.84[a]	0.02	3.79[a]	0.10	1.62[a]	0.01	2.05[a]	0.01
P-value effect of sex		0.03		0.005		0.0001		0.008		0.0001	

[a,b]Means that possess different superscripts differ significantly ($P \leq 0.05$).

[1]Starter, grower, and finisher diets were used to 21, 35, and 42 d of age, respectively.

[2]There were eight replicate pens of 25 birds each for each interaction mean except for the NC 2000 treatment after 35 d for which six replicate pens were used. Adjusted FCR includes the BW of birds that died during each diet period in the calculation.

[3]N7070 = Northrup King brand corn hybrid N7070; Bt = *Bacillus thuringiensis*; Liberty = glufosinate herbicide; NC2000 = North Carolina grown corn from 2000 growing season.

vs. the N7070-based isoline and transgenic corns) with respect to total protein (Table 2), it cannot be stated that the diets were identical (Table 3). It appeared that the NC2000 diets were not consumed as well as the N7070 series diets. It was possible that the NC2000 diet was of poorer pellet quality that would decrease feed intake, or that the NC2000 diet was more hygroscopic in the humid summer weather experienced at the time of the study, or both. These factors could affect the flow of the feed from the tube into the feeder pan, in spite of daily efforts to maintain equal feed flow by physical agitation of the feeders. Obvious evidence of this latter factor was found in two pens late in the finisher period, and these pens were deleted from the statistical analysis for the final time period and cumulatively. Therefore, the differences in FCR cannot necessarily be attributed to the corn source per se, but it is important to note that these data fail to show an obvious deleterious effect associated with the diets made from transgenic corn when compared to diets made from isoline and commercial corn. In a previous study with Event 176 Bt corn, Brake and Vlachos (1999) reported an advantage in FCR for birds fed Bt vs. non-Bt control hybrids. These authors suggested that the performance advantage for broilers fed Bt containing diets may have resulted due to lower mycotoxin levels for this corn. Other workers have identified no differences in FCR

TABLE 6. The effect of corn source and sex on survival of broiler chickens[1,2,3]

Corn source[4]	Sex	Starter deaths 0–21 d Mean	SEM	Grower deaths 21–35 d Mean	SEM	Finisher deaths 35–42 d Mean	SEM	Cumulative deaths 0–35 d Mean	SEM	Cumulative deaths 0–42 d Mean	SEM
		(%)									
N7070 Isoline		1.50	0.62	0.75	0.40	14.00	2.76	2.25	0.63	16.25	2.59
N7070Bt		0.50	0.34	0.25	0.25	18.25	4.87	0.75	0.40	19.00	4.95
N7070Bt + Liberty		1.25	0.48	0.75	0.40	14.25	3.41	2.00	0.63	16.25	3.43
NC 2000		1.75	0.63	0.50	0.34	6.57	1.81	2.25	0.73	9.14	2.39
P-value effect for corn source		0.39		0.69		0.09		0.22		0.23	
	Male	1.25	0.33	0.50	0.24	20.27[a]	2.98	1.75	0.40	22.13[a]	2.96
	Female	1.25	0.42	0.63	0.26	7.13[b]	1.24	1.88	0.47	9.00[b]	1.37
P-value effect for sex		1.00		0.72		0.0002		0.83		0.0003	
N7070 Isoline	Male	0.50	0.50	0.50	0.50	21.00	3.91	1.00[bc]	0.65	22.00	3.85
N7070 Isoline	Female	2.50	1.05	1.00	0.65	7.00	1.81	3.50[a]	0.91	10.50	2.13
N7070Bt	Male	1.00	0.65	0.50	0.50	27.50	7.84	1.50[abc]	0.73	29.00	7.77
N7070Bt	Female	0.00	0.00	0.00	0.00	9.00	3.98	0.00[c]	0.00	9.00	3.98
N7070Bt + Liberty	Male	1.50	0.73	0.00	0.00	20.00	6.05	1.50[abc]	0.73	21.50	6.18
N7070Bt + Liberty	Female	1.00	0.65	1.50	0.73	8.50	1.92	2.50[ab]	1.05	11.00	2.10
NC 2000	Male	2.00	0.76	1.00	0.65	10.00	3.39	3.00[ab]	1.00	14.00	3.97
NC 2000	Female	1.50	1.05	0.00	0.00	4.00	1.51	1.50[abc]	1.05	5.50	2.38
P-value effect for corn source × sex		0.20		0.06		0.54		0.04		0.58	

[a–c]Means that possess different superscripts differ significantly ($P \leq 0.05$).

[1]Starter, grower, and finisher diets were used to 21, 35, and 42 d of age, respectively.

[2]There were eight replicate pens of 25 birds each for each interaction mean.

[3]Survival expressed as percentage (%) deaths.

[4]N7070 = Northrup King brand corn hybrid N7070; Bt = *Bacillus thuringiensis*; Liberty = glufosinate herbicide; NC2000 = North Carolina grown corn from 2000 growing season.

TABLE 7. The effect of corn source on percentage carcass yield of male broiler chickens at 48 d of age[1,2]

	BW (g)		Dressed carcass[4] (%)		Fat pad (%)		Drums (%)		Thighs (%)		Wings (%)		Pectoralis major (%)		Pectoralis minor (%)	
Corn source[3]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
N7070 Isoline	2,922.5	63.78	72.99	0.37	1.21	0.07	11.03	0.12	13.40	0.23	7.80	0.06	14.95	0.32	3.90	0.05
N7070Bt	2,945.4	50.89	73.29	0.30	1.56	0.09	10.71	0.18	13.49	0.18	7.90	0.12	14.24	0.27	3.79	0.08
N7070Bt + Liberty	2,876.3	60.39	73.14	0.34	1.62	0.19	10.89	0.19	13.34	0.19	7.76	0.09	14.60	0.33	3.81	0.07
NC 2000	2,990.3	81.85	72.38	0.42	1.21	0.16	10.97	0.16	13.35	0.28	7.60	0.10	14.71	0.21	3.89	0.06
P-value	0.66		0.30		0.06		0.53		0.96		0.17		0.37		0.48	

[1]Starter, grower, and finisher diets were used to 21, 35, and 42 d of age, respectively.

[2]There were 16 birds processed within each corn source and sex.

[3]N7070 = Northrup King brand corn hybrid N7070; Bt = *Bacillus thuringiensis*; Liberty = glufosinate herbicide; NC2000 = North Carolina grown corn from 2000 growing season.

[4]Fresh, unchilled carcass from which the head, neck, feet, feathers, viscera and blood have been removed.

for broilers fed diets containing Bt and non-Bt isoline corn (Halle et al., 1998; Mirales et al., 2000; Piva et al., 2001; Taylor et al., 2001a,b). Gaines et al. (2001) reported finding differences in average daily feed intake and gain/feed (inverse of FCR) for broiler chickens fed diets containing corn from different genetic backgrounds, but no differences in these measures for groups fed Bt and non-Bt isoline hybrids with the same genetic background.

Survival

There were no significant differences in percentage survivors for birds that received the two transgenic corn diets and those that received the N7070 isoline and commercial NC2000 corn diets on an overall basis at any age (Table 6). However, there were significant differences due to sex, with males exhibiting higher mortality during the finisher phase (35 to 42 d) and cumulatively (0 to 42 d). This should be expected in extremely hot weather conditions as males are well known to be more susceptible to heat stress. There was a significant interaction of sex and corn source for the combined starter-grower periods (0 to 35 d) due to some erratic mortality during the grower period (21 to 35 d) that was not evident on a cumulative basis. This interaction did not follow any logical or explainable pattern and probably represents chance occurrences. Similarly, Brake and Vlachos (1999) identified no differences in survival for broilers fed Bt or control hybrids.

Carcass and Parts Yield

The yield of carcass parts as a percentage of live BW for males and females at 48 d of age are shown in Tables 7 and 8, respectively. There was no effect of corn source, which was consistent with results reported by Taylor et al. (2001a) for broilers fed Bt and non-Bt corn. However, Brake and Vlachos (1999) hypothesized that lower mycotoxin levels for Bt corn in their experiment may have explained the slight advantage in yield of pectoralis minor for birds fed diets containing Bt vs. isoline control hybrids.

Our study was carried out to determine whether diets prepared with transgenic corn would have any adverse effects on performance of broiler chickens when compared to diets prepared with nontransgenic (isogenic) control and commercial corn. Only minor differences were found due to corn source; performance was poorer for birds fed the commercial NC2000 corn diets. The transgenic N7070Bt and N7070Bt + Liberty hybrid diets supported broiler BW and FCR that was equivalent to growth from the N7070 isoline corn and better than that from the NC2000 corn without significant differences in carcass yield. While it is not clear whether the small differences in performance for NC2000 diets was attributable to the commercial corn per se or due to possible slight differences in overall composition of the formulated diets,

TABLE 8. The effect of corn source on percentage carcass yield of female broiler chickens at 48 d of age[1,2]

	BW (g)		Dressed carcass[4] (%)		Fat pad (%)		Drums (%)		Thighs (%)		Wings (%)		Pectoralis major (%)		Pectoralis minor (%)	
Corn source[3]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
N7070 Isoline	2,512.4	45.55	72.77	0.97	1.51	0.10	10.11	0.25	12.71	0.28	7.59	0.16	15.61	0.29	4.15	0.07
N7070Bt	2,423.1	70.41	73.00	0.40	1.86	0.18	10.33	0.14	12.70	0.17	7.99	0.10	15.30	0.30	4.17	0.10
N7070Bt + Liberty	2,387.9	36.49	73.30	0.36	1.77	0.12	10.33	0.12	12.81	0.14	7.86	0.08	15.28	0.25	4.19	0.06
NC 2000	2,393.4	36.89	73.09	0.28	1.66	0.06	10.36	0.12	12.98	0.14	7.73	0.09	15.35	0.22	4.13	0.07
P-value	0.26		0.93		0.24		0.70		0.72		0.07		0.80		0.94	

[1]Starter, grower, and finisher diets were used to 21, 35, and 42 d of age, respectively.

[2]There were 16 birds processed within each corn source and sex.

[3]N7070 = Northrup King brand corn hybrid N7070; Bt = *Bacillus thuringiensis*; Liberty = glufosinate herbicide; NC2000 = North Carolina grown corn from 2000 growing season.

[4]Fresh, unchilled carcass from which the head, neck, feet, feathers, viscera and blood have been removed.

it was clear that the transgenic corn had no deleterious effects in this study.

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Application for renewal of authorization of Bt11 maize and derived products notified according to Articles 11 and 23 of Regulation (EC) No 1829/2003 on genetically modified food and feed

PART I: TECHNICAL DOSSIER

APPENDIX 8.4

Utilization of Bt corn residues by grazing beef steers and Bt corn silage and grain by growing beef cattle and lactating dairy cows

Utilization of Bt corn residues by grazing beef steers and Bt corn silage and grain by growing beef cattle and lactating dairy cows[1]

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ABSTRACT: Three experiments were conducted to evaluate the impact of the *Bacillus thuringiensis* (Bt)-11 transformation event in two parental corn hybrids differing in date of maturity on beef and dairy cattle performance. Sixteen lactating Holstein dairy cows in replicated 4 × 4 Latin squares were assigned to four diets in a 2 × 2 factorial arrangement: Bt vs non-Bt trait and early- vs late-maturing corn hybrids. The diets contained 40% of the test corn silage plus 28% corn grain from the same corn hybrid (DM basis). There was no effect of the Bt trait on efficiency of milk production, ruminal pH, acetate:propionate ratio, or in situ digestion kinetics of NDF. The early-maturing corn hybrids resulted in greater total VFA concentrations in the rumen and efficiency of 4% fat-corrected milk production than the later-maturing hybrids ($P < 0.05$). Sixty-seven steer calves were used in a 70-d corn residue grazing trial for the late-maturing corn hybrids only. Daily BW gain of steers was similar for those grazing Bt and non-Bt corn residues, and the steers exhibited no grazing preference between Bt and non-Bt corn residue. One hundred twenty-eight steer calves were assigned to four silage-based growing diets in a 2 × 2 factorial arrangement: Bt vs non-Bt trait and early- vs late-maturing corn hybrids. The diets contained 90% corn silage and 10% supplement (DM basis). The DMI was higher for steers fed Bt compared with non-Bt hybrids ($P < 0.02$). An interaction ($P < 0.03$) was observed for feed efficiency between hybrid genotype and incorporation of the Bt trait. Feed efficiency was greater ($P < 0.05$) for steers fed the later-maturing non-Bt hybrid compared with the later-maturing Bt hybrid; however, feed efficiency was similar between steers fed early-maturing Bt and non-Bt corn silages. Steers fed the early-maturing hybrid gained 11% faster and were 7% more efficient compared with those fed the late-maturing hybrid. These latter results agree with the dairy experiment in which the early-maturing hybrid resulted in 5% greater efficiency of milk production than the later-maturing corn hybrid. In all experiments, incorporation of the Bt trait into corn had no consistent effect on cattle performance. In addition, background genetics of the corn hybrids appeared to have a more consistent impact on performance than did presence of the Bt trait.

Key Words: Beef Cattle, Dairy Cattle, Maize, Maize Byproducts, Maize Silage, *Bacillus thuringiensis*

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Introduction

Bacillus thuringiensis (**Bt**) is a naturally occurring soil bacterium that produces a protein that is toxic to some caterpillar (lepidopteran) insects, including European corn borer (Hyde et al., 1999). In this experiment, corn hybrids were used that contain a gene encoding for a Bt protein called ("Cry 1Ab" protein), which is one of several Bt-event hybrids that are commercially available. Transgenic corn plants used in this experiment that produce the Cry1Ab Bt protein provide protection against corn borer infestation throughout the life of the plant.

Bt corn hybrids are used for enhanced yields under conditions of borer pressure, which commonly occurs in the midwestern United States, and are becoming a widely accepted in the major corn-producing states. In many cases, the corn residue is used for growing or wintering beef cows and weaned calves. Corn silage is an important component of dairy feeding programs throughout the United States. Additionally, many beef calves are grown on silage-based diets in the fall and winter prior to finishing on high-grain diets in late winter and early spring. However, information on the feeding value of corn silage and corn residue from Bt hybrids compared with traditional hybrids is limited. Our hy-

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Table 1. Yield of grain, silage, and residual corn for experimental corn hybrids

	Early maturing		Late maturing	
Item	Bt[a]	Non-Bt	Bt	Non-Bt
Grain yield, kg/ha[b]	8,323	7,658	9,509	8,957
Grain yield, kg/ha[c]			11,549	11,424
Silage yield, kg/ha	31,608	27,125	36,315	39,453
Residual corn, kg/ha[d]			62.7	94.2

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b]Grain yield from nonirrigated field used for silage and grain production for beef growing and dairy lactation experiments.
[c]Grain yield from irrigated fields used for grain production and residue for cornstalk grazing experiment.
[d]Residual corn is amount of corn grain remaining in the field following grain harvest.

pothesis was that there would be similar performance between animals fed Bt and non-Bt corn.

Therefore, the objectives of this research were to 1) evaluate the effect of corn silage from two Bt corn hybrids (early- and late-maturing genotypes) and their respective near-isogenic counterparts on performance of growing beef steers, 2) evaluate the effect of corn residue from Bt and near-isogenic non-Bt corn hybrids on performance and grazing preference of growing beef steers, and 3) evaluate the effect of corn silage from the same two Bt corn hybrids and near-isogenic non-Bt hybrids on ruminal fermentation and short-term lactational performance of dairy cows.

Materials and Methods

Corn Cultivation, Harvest, and Chemical Composition

Four commercial corn hybrids (N4242Bt, N4242 non-Bt, N7333Bt, and N7333 non-Bt; Syngenta Seeds, Golden Valley, MN) were planted at the University of Nebraska Agricultural Research and Development Center located at Mead, NE in 1998. The Bt hybrids were derived from transformation event Bt11, and the non-Bt hybrids represented the corresponding near-isolines developed by conventional breeding methods. The N4242 hybrids were earlier-maturing (harvested for silage at 103 d), whereas the N7333 hybrids were later-maturing (harvested for silage at 112 d). All four hybrids were grown for harvest of silage in adjacent fields under comparable agronomic conditions without irrigation. At another site at the Research Center, under pivot irrigation, hybrids N7333 Bt and non-Bt were grown in adjacent fields for harvest of grain and used to conduct the corn residue grazing study. The seed drop rate per hectare for each hybrid at planting was as follows: N4242 Bt, 55,524; N4242 non-Bt, 56,950; N7333 Bt, 54,741; and N7333 non-Bt, 54,496. The yields of corn grain, corn silage, and residual corn in the crop residue for each hybrid are summarized in Table 1.

Agronomic characteristics, including plant height, number of ears, stalk and root lodging, and stalk diameter, were measured for all hybrids at 112 d after planting (three replicates of 10 plants each per field). These results are summarized in Table 2. Agronomic data were collected for all four hybrids grown without irrigation, in addition to the later-maturing Bt and non-Bt hybrids grown under irrigation for use in the stalk grazing experiment.

Each cornfield was evaluated for European corn borer infestation over a 5-d period on d 112, 113, and 116. For each field, 30 corn stalks (three replicates of 10 each) were evaluated for corn borer infestation. Samples were taken at least 10 rows from the field edge. The stalks were cut at the base and promptly dissected in the field. Leaf sheaths were removed and examined for feeding and for presence of larvae. The stalks were cut longitudinally to measure tunneling and to expose larvae. The ear and shank were examined also for larvae and measurement of tunnel length. Damage to individual plants was evaluated using the Guthrie visual rating scale (Guthrie et al., 1978). The corn borer pressure data are summarized in Table 3. These results apply to both irrigated (corn residue grazing experiment) and nonirrigated (corn silage feeding trials) fields.

The corn for silage was harvested at 3/4 milk line stage of maturity using a field chopper with knives adjusted to a 1-cm theoretical length of cut. For each hybrid, the chopped corn was ensiled in a separate Ag-Bag plastic silage bag (AgBag Int., Warrenton, OH) prior to the start of the beef and dairy experiments. Silage bags were opened and the feeding trials began a minimum of 100 d after ensiling. Grain was harvested at 85.3% DM for the earlier-maturing Bt and non-Bt hybrids (129 d after planting) and at 84.9% DM for the later-maturing hybrids (172 d after planting).

Corn Silage Chemical Composition. A weekly composite sample of each of the corn silage hybrids was created during the course of the animal experiments and was subsequently dried (60°C), ground (1-mm screen, Wiley mill, Arthur H. Thomas, Philadelphia, PA), and analyzed for CP (AOAC, 1990), ADF, NDF (Van Soest et al., 1991), acid detergent lignin (Goering and Van Soest, 1970), and permanganate lignin (Goering and Van Soest, 1970). Starch content of the silages was determined enzymatically (SDK Laboratories, Hutchinson, KS). Nitrogen fractions were measured according to the procedures of Licitra et al. (1996) to estimate the A, B_1, B_2, B_3, and C fractions for use in the Cornell Net Carbohydrate and Protein Model (1994).

The 30-h in vitro NDF digestibility was measured using ruminal fluid obtained from a grass-fed steer. Buffer solution was prepared as described by Goering and Van Soest (1970). From each composite sample created during the animal experiments, 300 mg of representative sample was taken and placed into 50-mL polypropylene tubes. There were two tubes per time point, and the entire in vitro experiment was replicated three times. The pH of ruminal fluid was 6.5 ± 0.3 for all replicates, and the temperature of the ruminal fluid

Table 2. Agronomic characteristics of silage fields measured 112 d after planting

Item	Early maturing		Late maturing	
	Bt[a]	Non-Bt	Bt	Non-Bt
Nonirrigated field				
Harvest population, plants/ha	54,363	51,892	54,363	51,892
Plant height, cm	244	236	274	277
Ears per 100 plants	92	92	96	96
Root lodged, %	0	0	0	0
Stalk lodged, %	0	3	0	0
Stalk diameter, cm	2.30	2.22	2.22	2.22
Irrigated field				
Harvest population, plants/ha			69,189	69,189
Plant height, cm			282	282
Ears per 100 plants			99	98
Root lodged, %			0	0
Stalk lodged, %			1	0
Stalk diameter, cm			2.38	2.54

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.

and buffer mixture was adjusted to 39°C. While each tube was being purged with CO_2, a 20% solution of ruminal fluid with buffer was dispensed in 30-mL aliquots using a Unispense II automatic dispensing machine (Wheaton Instruments, Millville, NJ). Tubes were sealed immediately with gas-release rubber stoppers. Samples were fermented for 30 h with gentle swirling occurring at 6-h intervals. Ash-free NDF was measured according to Van Soest et al. (1991). The IVDMD was measured using a modification of the original Tilley and Terry (1963) procedure. Briefly, a 48-h in vitro ruminal fermentation was carried out as described above. Then, the residue was incubated with pepsin and HCl for 24 h at 30°C. The inoculum was composed of a 1:1 mixture of ruminal fluid plus buffer.

Dairy Lactation Experiment

Twelve intact (four primiparous, eight multiparous) and four ruminally fistulated, multiparous Holstein dairy cows were assigned to one of four treatments in a replicated 4×4 Latin square design with 3-wk periods. Cows averaged 162 ± 10 d in milk when they were assigned to treatments. Cows were not injected with bovine somatotropin during this experiment. Dietary treatments were arranged as a 2 × 2 factorial of corn hybrid (early- vs later-maturing) and Bt trait. The diets contained (DM basis) 10% alfalfa silage, 40% of the test corn silage, 28% rolled corn grain, and 22% of a concentrate mixture composed of soybean meal, blood meal, tallow, minerals, and vitamins. The diets were designed so that the corn grain and corn silage were from the same hybrid for each diet to maximize any possible effect of the Bt trait on animal response. The ingredient composition of the experimental diets is shown in Table 4. All diets were formulated to contain approximately 17.5% CP and to meet the metabolizable protein requirement as predicted by the Cornell Net Carbohydrate and Protein Model (1994). The four diets were fed as total mixed rations twice daily in amounts to ensure a minimum of 10% refusals.

Table 3. Summary of Guthrie scale ratings and other indicators of larval infestation (irrigated and nonirrigated fields)

Item	Early maturing		Late maturing	
	Bt[a]	Non-Bt	Bt	Non-Bt
Number of observations	30	30	60	60
% Infestation[b]	0	33	0	56
Larvae/plant	0	1.8	0	0.6
Average tunnel lengths, cm				
Stalk	0	5.1	0.025	1.5
Ear shank	0	0.57	0	0.12
Ear	0	0.33	0.025	0.25
Guthrie rating[c]	1.0	2.3	1.0	1.4

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b]Infested with live larvae (% of stalks).
[c]As calculated by Guthrie et al. (1978). Guthrie Scale = 1 no or minimal leaf damage to 9 severe leaf damage.

Experimental periods were 21 d, with the last 7 d used for sample and data collection. Cows were housed in a tie-stall barn equipped with individual feeding boxes. Cows were removed from the barn for milking, exercise, and estrus detection twice daily for a total of approximately 4 h.

Daily milk yields were recorded electronically. Composite a.m. and p.m. milk samples were collected for 2 d at the end of each period and analyzed for fat, protein, and lactose (Milko-Scan Fossomatic; Foss Food Technology Corp., Eden Prairie, MN). Calculation of milk composition was weighted according to a.m. and p.m. milk yields. Body weight was measured weekly immediately following the a.m. milking.

For fistulated cows only, samples of ruminal fluid were collected during the last week of each period by collecting fluid immediately beneath the ruminal mat at 0, 6, 12, 18, and 24 h after feeding. Ruminal pH was

Table 4. Ingredient and chemical composition of experimental diets used in dairy experiment (% of DM)

	Early maturing		Late maturing	
Item	Bt[a]	Non-Bt	Bt	Non-Bt
Ingredient				
Alfalfa silage[b]	10.0	10.0	10.0	10.0
Corn silage	40.0	40.0	40.0	40.0
Rolled corn grain	28.0	28.0	28.0	28.0
Soybean meal (46.5% CP)	17.9	17.9	18.3	18.3
Blood meal	0.67	0.67	0.69	0.69
Tallow	0.57	0.57	0.57	0.57
Mineral and vitamin mix[c]	2.86	2.86	2.44	2.44
Composition				
DM, %	52.3	52.3	52.8	52.8
CP	17.5	17.5	17.3	17.3
RUP[d]	6.31	6.32	6.27	6.29
ADF	16.6	15.3	17.4	16.0
NDF	26.3	25.5	27.0	27.8
NE_L, Mcal/kg[d]	1.68	1.68	1.68	1.68

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b]Chemical composition of first-cutting alfalfa silage harvested at bud stage was (DM basis): DM, 45.1%; CP, 21.9%; ADF, 35.0%; and NDF, 44.8%.
[c]Supplement contained (DM basis) 15.2% Ca, 7.2% P, 4.1% Mg, 4% Na, 3,000 mg/kg of Zn, 1,750 mg/kg of Mn, 400 mg/kg of Cu, 200,000 IU/kg of vitamin A, 36,000 IU/kg of vitamin D_3, and 600 IU/kg of vitamin E.
[d]Calculated using nutrient composition and digestibility data from Tables 6 and 7 plus data in NRC (1989).

measured using a portable pH meter, and samples were prepared to determine VFA concentration using GLC (Erwin et al., 1961). The VFA samples were analyzed using a chromatograph (model 5890; Hewlett Packard, Wilmington, DE) with a 2-mm i.d. column that was 2.4 m in length and packed with SP 1200 (Supelco, Bellefonte, PA). The rate of N_2 flow was 20 mL/min, injection temperature was 170°C, column temperature was 120°C, and the flame ionization detector temperature was 200°C.

Fractional rate of NDF digestion of each corn silage hybrid was determined using the in situ bag technique. Dacron bags (Ankom, Fairport, NY) containing 5 g of substrate were incubated in duplicate within the rumen of each fistulated cow for 0, 6, 12, 18, 24, 36, 48,72, and 96 h. Bags were 10 × 20 cm with a mean pore size of 53 μm. Prior to ruminal incubation, dried corn silage samples were ground through a 2-mm screen using a Wiley mill. After removal from the rumen and rinsing (Wilkerson et al., 1995), all bags were dried at 60°C and weighed. Contents were analyzed for ash-free NDF (Van Soest et al., 1991), and values within time were pooled. The kinetics of ruminal NDF digestion were calculated as described by Grant (1994).

Performance data for cows were analyzed as a replicated 4 × 4 Latin square design with model effects for square, cow within square, period, treatment, square × treatment, and residual error using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Orthogonal comparisons were performed for early- vs later-maturing hybrids, Bt vs non-Bt trait, and the interaction of background genotype and Bt trait. Unless otherwise stated, significance was declared at $P < 0.05$.

Corn Residue Grazing Experiment

Sixty-seven large-framed steer calves (284 ± 3.7 kg) were used in a two-part, 70-d grazing trial. The total area available for grazing contained 11.3 ha of crop residue from the later-maturing non-Bt and 12.1 ha of residue from the later-maturing Bt corn hybrids. The two fields of corn residue were divided into six pastures (three Bt and three non-Bt) and then stocked with 51 steers. To achieve equal stocking rates (0.28 animal units/ha), the three non-Bt pastures were each assigned eight steers and the three Bt pastures were each assigned nine steers. These six pastures accounted for 8.5 and 9.3 ha of the non-Bt and Bt corn residue, respectively. The remaining 2.8 hectares of Bt and non-Bt residue were used for a grazing preference experiment. Steer BW was measured for two consecutive days, at the start and finish of the trial, and after a 3-d period of limit-feeding (2% of BW) to minimize fill effects. Steers received a protein supplement at approximately 0.68 $kg \cdot steer^{-1} \cdot d^{-1}$ to ensure that protein intake did not limit performance while grazing the corn residue.

Prior to grazing, residual corn (kg/ha) was estimated by counting full and partial ears from one row (76 cm width) for 30.5 m at six locations in each of the six pastures. Corn kernels were then removed from the cob and weighed to determine the amount of corn remaining in the field for each hybrid. Residue samples were collected three times during the grazing period to reflect the entire grazing period. Samples were collected on d 5, 36, and 72, by gathering all residue (corn, leafs, husks, and stalk) from one row (76 cm width) for 3.5 m at two locations in each of the pastures and analyzed for IVDMD. Although grazing cattle do not typically consume actual corn stalks, corn stalk samples were used to determine stalk-breaking strength in an attempt to discover any physical differences that would deter grazing. The corn stalk samples were collected at the same time as the residue samples by cutting the stalk between the root crown and the first node, in two locations in each of the pastures. Corn stalks were then dried in a 60°C oven 24 h prior to measuring breaking strength. An INSTRON 5500R Universal Testing Machine (Instron, Canton, MA) equipped with a 2,500-kg load cell and a crosshead speed of 50 mm/min was used to measure the total energy required to break the stalk (Bourne, 1978). A blunt wedge was used to break the corn stalk after it was placed on a bone breaking bridge with a width of 9 cm and a depth of 4 cm.

The steer BW and gain data were analyzed as a randomized complete block design. Pasture was the experimental unit, resulting in three replications for Bt and non-Bt treatments. Least squares means (SAS Inst. Inc., Cary, NC) were used to compare ADG of steers grazing Bt and non-Bt corn residue. Residual error was used to calculate the F-test for determining the significance of treatment effects.

The second component of the stalk grazing experiment evaluated grazing preference for Bt and non-Bt corn residue. Sixteen steers grazed one pasture containing equal areas (2.8 ha) of Bt and non-Bt corn residue for 70 d. These steers also received 0.68 kg of protein supplement daily on a per-steer basis. Animals were observed once daily to collect grazing preference data, and numbers of animals grazing Bt and non-Bt stalks were recorded. All measurements were collected prior to supplementation between 0600 and 1000 daily. There were a total of 50 observations made during the 70-d grazing period. On the remaining 20 d, all steers were observed in the supplementation areas or near water tanks rather than grazing. Additionally, snow cover prevented grazing for a portion of these 20 d. The entire 20 d were removed from the analysis, and only the days that the steers were observed grazing were included. The percentage of steers grazing Bt and non-Bt hybrids was analyzed using the PROC GLM procedure of SAS (SAS Inst. Inc.). Residual error was used to calculate an F-test for least square mean separation.

Table 5. Experimental diets for beef steer growing experiment using corn silage (% of DM)

	Early maturing		Late maturing	
Item	Bt[a]	Non-Bt	Bt	Non-Bt
Ingredients				
Corn silage	90.0	90.0	90.0	90.0
Supplement	10.0	10.0	10.0	10.0
Supplement ingredients				
Soybean meal (46.5% CP)	65.0	65.0	75.0	75.0
Dry-rolled sorghum	11.6	11.6	0	0
Urea	8.50	8.50	10.0	10.0
Limestone	8.23	8.23	8.47	8.47
Salt	3.0	3.0	3.0	3.0
Tallow	2.2	2.2	2.2	2.2
Dicalcium phosphate	1.02	1.02	0.86	0.86
Vitamin and mineral mix[b]	0.47	0.47	0.47	0.47
Chemical composition				
DM, %	37.3	37.3	37.3	37.3
CP	12.4	12.4	12.3	12.3
NDF	35.0	33.1	37.0	38.2
ADF	22.2	19.9	23.7	21.4
Ca	0.60	0.60	0.61	0.61
P	0.25	0.25	0.25	0.25

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b]Mix was formulated to meet or exceed nutrient requirements of NRC (1996).

Corn Silage Beef Growing Experiment

One hundred twenty-eight medium-framed steer calves (282 ± 16 kg) were used in a completely randomized design with a 2 × 2 factorial arrangement of treatments. Steers were stratified by initial BW and assigned randomly to pens to ensure that the initial starting weight and coefficient of variation for each pen were similar. A total of 16 pens were used with eight steers per pen, yielding four replications per treatment. Steers were implanted on d 1 with Ralgro Magnum (Mallinckrodt Veterinary, Mundelein, IL; 72 mg of Zeranol) and fed diets for 101 d. During this time steers were allowed ad libitum access to treatment diets and water. Initial and final BW were measured on two consecutive days following a 3-d period of limited feed intake (approximately 2% of BW) to minimize fill effects. Additionally, interim BW were measured at 35-d intervals to monitor animal performance. Steers used in this experiment had been vaccinated against bacterial and viral diseases and treated for internal and external parasites approximately 2 mo prior to initiation of the experiment.

The diets (Table 5) contained 90% corn silage and 10% supplement (DM basis). A blend of soybean meal and urea (75:25% N basis) was used as the protein supplement. Because the silage hybrids varied slightly in CP concentration, supplements were formulated to be equal across treatments to meet NRC requirements for MP (Table 5). Finely ground grain sorghum was included at 11.6% (DM basis) of the supplement for the diets containing earlier-maturing corn to equalize dietary MP requirements. This amount of inclusion in the supplement resulted in 1.2% of the diet as grain sorghum. All diets contained 20 g/t Rumensin (monensin sodium).

Data were analyzed as a completely randomized design with a 2 × 2 factorial arrangement of treatments. Pen was the experimental unit. Orthogonal contrasts were used to compare early- vs later-maturing hybrids, Bt vs non-Bt trait, and the interaction of corn hybrid genotype and Bt trait. Residual error was used for testing orthogonal contrasts and treatment effects. When interactions were present, the PDIFF procedure of SAS (SAS Inst. Inc.) was used to separate differences among the four treatments. Unless otherwise stated, significance was declared at $P < 0.05$.

All animals in this series of experiments were managed using protocols approved by the Institutional Animal Care and Use Committee at the University of Nebraska.

Results and Discussion

Corn Borer Pressure

The Bt11 event results in endotoxin production in all plant tissues providing 98% control of first and second, generation corn borers (Rice and Pilcher, 1998). Results of the larval infestation evaluations (Table 3) indicated that the plants without Bt protection did incur some degree of corn borer infestation relative to the Bt hybrids (0 vs 33 and 56% infestation). The amount of infestation was unexpectedly high given the generally low population levels for European corn borer observed throughout much of Nebraska in 1998. Results from strip trials conducted at six sites in Nebraska during

Table 6. Nitrogen and protein fractions of experimental silages used in beef and dairy experiments

Item	Early maturing		Late maturing	
	Bt[a]	Non-Bt	Bt	Non-Bt
DM%	40.2	39.0	37.6	37.8
	% of DM			
CP	6.82	6.68	6.44	6.51
Nonprotein nitrogen	3.42	3.17	3.13	3.49
True protein	3.41	3.51	3.31	3.02
True soluble protein	0.33	0.34	0.48	0.17
Insoluble protein	3.08	3.18	2.83	2.85
NDIP[b]	1.34	1.39	1.07	1.10
ADIP[c]	1.11	0.90	0.84	0.91
Protein fraction[d]				
A	3.42	3.17	3.13	3.49
B_1	0.33	0.34	0.48	0.17
B_2	1.74	1.89	1.76	1.76
B_3	0.23	0.39	0.23	0.19
C	1.11	0.90	0.84	0.91

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b]Neutral detergent insoluble protein.
[c]Acid detergent insoluble protein.
[d]Protein fractions measured according to the procedures of Licitra et al. (1996).

Table 7. Carbohydrate and lignin composition of experimental silages and grain used in beef and dairy experiments

Item	Early maturing		Late maturing	
	Bt[a]	Non-Bt	Bt	Non-Bt
Corn silage				
DM, %	40.2	39.0	37.6	37.8
	% DM			
Ash	4.1	4.5	6.1	4.7
ADF	25.0	21.9	26.9	23.9
NDF	38.9	36.7	41.1	42.4
ANDF[b]	38.0	36.2	38.6	41.2
ADL[c]	3.25	2.69	3.62	3.36
PL[d]	4.90	4.14	5.26	5.04
Starch	37.6	38.6	37.3	37.1
30-h NDF digestion, %[e]	32.4	30.8	34.4	31.6
IVDMD, %[f]	74.3	65.6	69.1	65.6
Corn grain				
DM, %	85.8	85.9	85.2	85.4
CP	7.1	7.0	6.0	6.1
Starch	76.7	74.5	76.5	76.6

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b]NDF corrected for ash content according to Van Soest et al. (1991).
[c]Acid detergent lignin measured according to Goering and Van Soest (1971).
[d]Permanganate lignin measured according to Goering and Van Soest (1971).
[e]Measured in vitro.
[f]In vitro dry matter digestibility measured using modified procedures of Tilley and Terry (1963) as described in text.

1998 indicated an infestation rate of only 11% (B. Siegfried, University of Nebraska Entomology Department, personal communication).

Determination of Cry 1Ab Protein Concentrations

The Cry 1Ab protein content was measured using an enzyme-linked immunosorbent assay (Syngenta Seeds, Research Triangle Park, NC) for the freshly chopped, pre-ensiled corn plant, the silages, and stalk residue (only hybrid 7333, which was used in the grazing study). The Cry1Ab protein was detected in the fresh, pre-ensiled Bt plant material at concentrations of 4,923.5 and 8,508.8 ng Cry1Ab/g dry weight for N4242Bt and N7333Bt, respectively. For hybrid 4242Bt, Cry 1Ab content declined to trace, unquantifiable amounts by 9 d after ensiling, whereas for hybrid 7333Bt, Cry1Ab content declined to trace amounts by 4 d after ensiling. Stalk samples for hybrid 7333 used in the grazing study had a Cry1Ab content of 935.9 ng/g dry weight after harvest, compared with 590.2 ng/g 3 mo later during the grazing study. There was no detectable Cry1Ab protein in 7333 non-Bt stalks.

Chemical Composition and Digestibility of Corn Hybrids

The chemical composition and digestibility of the experimental silages obtained from the weekly composite samples are detailed in Tables 6 and 7. The later-maturing corn hybrids contained less CP than the earlier-maturing hybrids, although the protein fractions were similar among hybrids. Additionally, the earlier-maturing hybrids contained less NDF and ADF than the later hybrids, which likely reflected a greater starch and CP content. Lignin, measured as either permanganate lignin or as acid detergent lignin, was slightly greater for the Bt vs non-Bt hybrids and was substantially lower for the earlier- vs later-maturing hybrids. Starch content and IVDMD were greater for the earlier- vs later-maturing hybrids. There was very little difference among the hybrids in 30-h in vitro NDF digestibility, although the Bt hybrids were consistently higher than non-Bt hybrids. The relative lack of chemical composition differences between the Bt and non-Bt hybrids from transformation event Bt11 agrees with the data of Faust and Spangler (2000), who evaluated the nutritive value of several MON810 Bt and near-isogenic corn hybrids and observed no differences among the hybrids. These observations indicate that Bt corn silage should have a feeding value similar to that of non-Bt corn silage.

Dairy Lactation Experiment

Feed Intake, Milk Production, and Milk Composition. There was no effect of either maturation date or Bt vs non-Bt trait on DMI in kilograms per day or as a percentage of BW (Table 8). Daily DMI averaged 22.8 kg/d or 3.77% of BW. Average BW (618 kg) and change in BW per 21-d period (20.8 kg) did not differ among treatments. Due to the short duration of experimental periods (21 d), body condition score was not measured.

Table 8. Effect of Bt[a] vs non-Bt corn hybrid on short-term performance of lactating dairy cows

	Early maturing		Late maturing		
Item	Bt	Non-Bt	Bt	Non-Bt	SEM
DMI					
kg/d	22.8	22.4	23.2	22.7	0.1
% of BW	3.75	3.72	3.84	3.75	0.02
BW					
kg	619	615	615	621	3.0
Change per 21-d period	21.4	22.7	21.1	18.0	1.9
Milk, kg/d	29.2	28.6	28.7	28.5	0.3
Milk fat					
%	3.80	3.82	3.70	3.73	0.06
kg/d	1.11	1.09	1.06	1.06	0.02
Milk protein					
%	3.54	3.55	3.51	3.52	0.02
kg/d	1.03	1.01	1.01	1.00	0.01
Milk lactose					
%	4.90	4.85	4.87	4.80	0.40
kg/d	1.43	1.38	1.40	1.37	0.04
4% Fat-corrected milk (FCM), kg/d	28.3	27.7	27.4	27.3	0.5
FCM/DMI, kg/kg[b]	1.26	1.24	1.19	1.20	0.03

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b]Significant effect of early versus late-maturing corn hybrid ($P < 0.05$).

As with DMI, there was no influence of corn hybrid or Bt trait on milk production. There was a range of only 0.6 kg/d in milk production per cow across all four treatments. Average milk production was 28.8 kg/d.

Milk protein percentage, and most importantly, milk protein production were unaffected by treatment (Table 8). Milk protein percentage averaged 3.53%, and milk protein production averaged 1.01 kg/d. Likewise, milk lactose percentage (4.86%) and lactose production (1.40 kg/d) were similar among diets. There was no effect ($P > 0.05$) of earlier- vs later-maturing corn hybrid on milk fat percentage. In summary, there was no effect of Bt vs non-Bt trait on any measure of milk composition.

Efficiency of 4% fat-corrected milk production (FCM/DMI, kg/kg) was greater for the earlier- vs later-maturing hybrids ($P < 0.05$). This response likely reflected the higher digestibility and lower fiber content of the earlier-maturing hybrid. There is a considerable body of literature that shows the relationship between variation in composition and digestibility of corn silage and variation in milk production and efficiency as summarized by Johnson et al. (1999). Again, there was no effect, or any interactions, of Bt vs non-Bt trait on production of FCM or efficiency of FCM production.

Ruminal pH, VFA, and NDF Digestion Kinetics. The effect of corn silage hybrid on ruminal pH and VFA

Table 9. Effect of corn silage hybrid on ruminal pH and VFA concentrations in lactating dairy cows[a]

	Early maturing		Late maturing		
Item	Bt[b]	Non-Bt	Bt	Non-Bt	SEM
Ruminal pH[c]	5.87	5.67	5.81	5.82	0.06
Total VFA, m*M*[d]	97.2	93.8	83.2	85.0	1.7
Acetate (A)	55.1	57.2	53.3	51.3	<0.1
Propionate (B)	21.3	20.3	16.9	19.6	<0.1
n-Butyrate	9.9	11.5	9.6	10.0	<0.1
Isobutyrate	1.05	1.19	0.96	1.02	<0.1
n-Valerate	1.33	1.61	1.06	1.35	<0.1
Isovalerate	1.69	1.97	1.38	1.66	<0.1
A:P	2.8	2.9	3.1	3.1	0.1

[a]Results obtained from fistulated cows.
[b]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[c]Mean of samples collected every 4 h for 24 h.
[d]Significant effect of early- vs later-maturing corn hybrid ($P < 0.05$).

Table 10. In situ NDF digestion kinetics of corn silage hybrids measured in lactating dairy cows

	Early maturing		Late maturing		
Item	Bt[a]	Non-Bt	Bt	Non-Bt	SEM
Lag, h[b]	5.96	8.89	6.19	6.26	0.74
K_d, h[c]	0.034	0.035	0.038	0.033	0.005
Extent, %[d]	57.6	57.4	64.6	57.2	0.44

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b]Discrete lag time prior to NDF digestion.
[c]Fractional rate of NDF digestion.
[d]Potential extent of NDF digestion at 96 h of fermentation.

concentrations is summarized in Table 9. There were no significant interactions ($P > 0.05$) between time of ruminal fluid sampling and diet on ruminal pH or VFA; therefore, means over 24 h are presented. Ruminal pH was unaffected by treatment and averaged 5.79. Total VFA concentration was greater for the earlier-maturing Bt and non-Bt hybrids compared with the later-maturing Bt and non-Bt hybrids. There was no effect of the Bt trait itself on total VFA concentration or acetate:propionate ratio.

There was no effect of the Bt trait or hybrid background genotype on in situ digestion kinetics of NDF (Table 10). The fractional rate of NDF digestion averaged 0.035 h^{-1}, and the potential extent of NDF digestion averaged 59.2%. These data support the in vitro 30-h NDF digestion data, which indicate little difference in NDF digestion between Bt and non-Bt hybrids.

Corn Residue Grazing Experiment

Performance of steers grazing corn residue is presented in Table 11. There was no effect ($P = 0.12$) of the Bt trait on daily gain of steers. Similarly, Russell et al. (2000) observed no deleterious effects on gestating beef cows when grazing Bt corn residue. Previous Nebraska research has demonstrated a positive correlation ($r = 0.79$) between residual corn and daily BW gain (Jordon et al., 1997). Gains observed in our study were lower than those often observed with calves grazing corn residues, typically 0.40 to 0.54 kg/d (Clanton, 1989; Jordan et al., 1997). As a result of low European corn borer pressure and good harvest conditions, the amount of residual corn was lower than normally observed at this location, typically 2 to 4% of corn yield. The amount of residual corn was 62.7 and 94.2 kg/ha for the Bt and non-Bt hybrids, respectively. These amounts correspond to 0.5 and 0.8% of corn yield for the Bt and non-Bt hybrids, respectively (Table 1).

The IVDMD of the Bt corn residue was 3 percentage units lower ($P = 0.04$) than the residue from non-Bt hybrids (Table 11). This small change in IVDMD, although statistically significant, appeared to have little impact on animal performance. As mentioned previously, performance of steers grazing corn residue is highly correlated to the amount of residual corn remaining in the field. Moreover, there were no significant effects of corn residue type (Bt vs non-Bt) on steer performance in this experiment. The IVDMD of the residue in this study is quite low in comparison to previous research (Fernandez-Rivera and Klopfenstein, 1989) due to our sampling technique of gathering total residue including unselected, poor-quality portions of the residue. The amount of energy required to break the corn stalk was similar ($P = 0.13$) for Bt and non-Bt corn stalks. Although animals grazing stalks rarely consume the stalks (Fernandez-Rivera and Klopfenstein, 1989), we wanted to preclude differences in stalk strength as a potential reason for preference of Bt or non-Bt residue.

The distribution of steers grazing Bt and non-Bt corn residue was similar (F-test; $P = 0.51$). On average, 47.5% of the steers were observed grazing Bt corn residue, and 52.5% of the steers were observed grazing non-Bt residue. Similarly, Hendrix et al. (2000) found no effect of the Bt trait on corn residue grazing preference for beef steers.

Table 11. Performance and grazing preference of growing steers grazing Bt and non-Bt corn residue (later-maturing hybrid only)

Item	Bt[a]	Non-Bt	SEM	P-value
Performance				
Initial BW, kg	284	284	0.35	0.89
Final BW, kg	301	306	2.0	0.20
ADG, kg/d	0.24	0.32	0.03	0.12
IVDMD, %[b]	33.0	36.0	0.7	0.04
Energy required to break stalk, mJ[c]	2,687	2,349	152	0.13
Grazing preference distribution[d]	47.5	52.5	5.2	0.5

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b]In vitro dry matter digestibility measured using modified procedures of Tilley and Terry (1963) as described in text.
[c]Total energy required to break corn stalks measured by an INSTRON Universal Testing Machine.
[d]Percentage of steers observed grazing Bt or non-Bt corn residue.

Corn Silage Beef Growing Experiment

Results for the silage growing study are summarized in Table 12. Feeding Bt corn hybrids resulted in higher ($P = 0.02$) DMI than feeding near-isogenic non-Bt hybrids. An interaction ($P < 0.05$) was observed for ADG and feed efficiency between background genotype of the hybrid (early vs later maturity) and the presence of the Bt trait. Daily gain was higher ($P < 0.05$) for steers fed the earlier-maturing Bt compared with the earlier-maturing non-Bt hybrids. However, ADG was similar between steers fed later-maturing Bt and non-Bt hybrids. In contrast, feed efficiency was better ($P < 0.05$) for steers fed later-maturing non-Bt compared with later-maturing Bt hybrids. Feed efficiency was similar between steers fed earlier-maturing Bt and non-Bt hybrids. Although an interaction was observed for ADG and feed efficiency, overall, steers fed the earlier-matur-

Table 12. Performance (101 d) of growing beef steers fed experimental corn hybrids as silage

	Early maturing		Late maturing			P-values		
Item	Bt[a]	Non-Bt	Bt	Non-Bt	SEM	Bt	Hybrid	Bt × hybrid
Initial BW, kg	281	282	281	282	<1	0.08	0.88	0.93
Final BW, kg	428[b]	419[bc]	407[d]	413[bc]	3	0.56	0.002	0.04
DMI, kg/d	8.71	8.42	8.51	8.22	0.11	0.02	0.09	0.96
ADG, kg	1.46[b]	1.36[c]	1.25[d]	1.30[bc]	0.03	0.39	<0.001	0.03
Gain/feed, kg/kg	0.167[b]	0.161[bc]	0.147[d]	0.158[b]	0.003	0.43	0.001	0.007

[a]Bt = *Bacillus thuringiensis* (Bt)-11 transformation event.
[b,c,d]Means within the same row not bearing a common superscript differ ($P < 0.05$).

ing hybrids gained 11% faster ($P < 0.01$) and were 7% more efficient ($P < 0.01$) than those fed corn silage produced from the later-maturing hybrids. The interaction of corn hybrid and incorporation of the Bt gene is difficult to explain, but was likely related to differences in silage chemical composition (NDF, ADF, lignin, starch; Table 7).

Overall Corn Hybrid Effects

For both the beef and dairy experiments, the earlier-maturing hybrids resulted in greater efficiency of production than the later-maturing hybrids. An explanation for this response may be found by comparing several of the compositional differences between the two hybrids. Although we could not measure a difference in NDF digestibility between the two hybrids, when comparing them, we observed that the earlier-maturing hybrid had 10.6% lower NDF content, 17.5% less acid detergent lignin, 2.5% higher starch content, 9.8% higher nonfiber carbohydrates, and 4.2% higher IVDMD. Our conclusion is that the increased animal performance in the silage growing experiment reflects the cumulative benefits of all of these compositional changes. Data generated at Kansas State University (Bolsen, 1992), which summarized several years of silage comparisons, concluded that these cumulative changes in chemical composition, even though some are relatively small, such as starch, could explain observed differences in animal performance.

Overall Bt Trait Effects

Incorporation of the Bt trait into two different background genotypes had no influence on performance or grazing distribution of steers grazing corn residue or lactating dairy cows consuming corn silage and corn grain. The interaction observed between hybrid genetics and incorporation of the Bt trait for growing steers consuming corn silage-based diets is difficult to explain, and is most likely related to slight changes in the chemical composition of the silages. Most importantly, the background genetics of the hybrids selected for incorporation of the Bt gene had a much larger influence on daily gain and feed efficiency of growing steers fed corn silage-based diets than did incorporation of the Bt gene into these hybrids.

Implications

Data from these experiments suggest that the Bt trait had no effect on the preference for or performance of steers grazing Bt or non-Bt corn residue. Incorporation of the Bt gene into these two background genotypes resulted in different effects on the feeding value of the silage for growing steers, but no difference for lactating dairy cows. The Bt corn had no measurable impact on short-term lactational performance of dairy cows and did not negatively affect ruminal fiber degradation. Across these beef and dairy experiments, incorporation of the Bt trait into corn had no consistent effect on animal performance. However, parental hybrid genotype affected efficiency of animal production similarly in both the beef and dairy experiments. Producers can take advantage of increased yields and reduced pesticide use with Bt corn hybrids without adversely affecting corn residue grazing or use of silage and grain for growth and lactation.

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Application for renewal of authorization of Bt11 maize and derived products notified according to Articles 11 and 23 of Regulation (EC) No 1829/2003 on genetically modified food and feed

PART I: TECHNICAL DOSSIER

APPENDIX 8.5

Effects of feeding calves genetically modified corn Bt11: a Clinico-Biochemical study

NOTE *Clinical Pathology*

Effects of Feeding Calves Genetically Modified Corn Bt11: A Clinico-Biochemical Study

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ABSTRACT. Genetically modified corn Bt11 is insect-resistant and expresses Cry1Ab toxin, an insecticidal protein, in kernels. Although Bt11 corn is considered safe based on animal performance, there are no reports available on the clinico-biochemical effects of feeding it to cattle. In this study, we evaluated the effects of feeding Bt11 to calves, using blood and ruminal clinico-biochemical parameters. Our three-month-long feeding experiment demonstrated that calves (n=6), fed with a ration containing 43.3% of Bt11 corn kernels as dry matter, did not develop any discernible clinical, hematological, biochemical, or ruminal abnormalities as compared with control calves (n=6) fed non-Bt11 corn. The results suggest that the transgenic Bt11 has no negative clinico-biochemical effects on calves.

KEY WORDS: Bt11, calf, clinico-biochemical parameters.

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The use of biotechnology in insect-resistant plants greatly contributes to the efficiency of crop production and is expected to be a key technology for resolving breadbasket issues or global environment problems. However, there are public concerns about the ecological risks and safety of the byproducts of these transgenic crops.

Bt11 corn, a transgenic insect-resistant plant designed to produce insecticidal Cry1Ab toxin, provides specific protection against Lepidoptera infestation throughout the life of the plant. The toxin is lethal to the target insect in that it damages the insect's mid-gut cells. The toxin is activated by the mid-gut protease when ingested by the susceptible insect larvae. Following that, the Cry toxin is considered to attack the insect's mid-gut epithelial cells by binding to receptors on the cells, invading the cell membrane, and forming somewhat cation-selective ion channels that result in the disintegration of the membrane and osmotic cell lysis. Based on *in vivo* experiments [6, 7], the Cry toxin, however, is considered harmless to humans and farm animals due to the lack of both the activation process of Cry proteins [9] and the Cry toxin receptor in mammalian intestinal epithelial cells [10, 12]. Consequently, the Cry toxin has become a genetic resource for developing insect-resistant, genetically modified (GM) plants including Bt11 [2]. Before the introduction of Bt11 corn, there were several studies to assess its safety based on livestock performance [1, 4, 5]. However, there is little knowledge available on the clinico-biochemical effects of feeding Bt11 to cattle. Therefore, in this study, we examined the health status, hematological, biochemical and rumen functions of calves fed Bt11 corn for three months.

Bt11 (N58-D1 Lot#2608611) and non-Bt isoline (NX5768 Lot#2608612) were purchased from Novartis Seed Inc. (NC, U.S.A.). The purity of Bt11 was 99%, and the contamination to non-Bt isoline was 1.3%, which were validated by Genetic ID Inc. (Kanagawa, Japan). Novartis Seed Inc. also confirmed that there was no contamination of mycotoxins (Aflatoxin B1, B2, G1, G2, Zearalenone, Deoxynivalenol and Fumonisin B1, B2, B3). Corn meal was prepared using a hammer mill attached to a 3 mm sieve. The experimental feeds contained 43.3% Bt11 or non-Bt corn kernel as dry matter. The composition of the experimental feed [3] was determined according to the Japanese feeding standard for beef cattle (Agriculture, Forestry and Fisheries Research Council Secretariat, MAFF, Tokyo, Japan, 2000) to attain a daily body weight gain of 1 kg.

Twelve healthy 2-month-old cross-breed calves (Japanese Black × Holstein) were housed individually for three weeks for acclimatization. A ruminal fistula was surgically inserted in each calf, and the calves were housed another 3 weeks for recovery. The calves were then randomly assigned to experimental (Bt11-fed) and control (non-Bt-fed) groups of 6 calves each and reared for 3 months. This experimental duration was set to meet the minimum period for safety assessment of a GM feed [6]. The feed was supplied twice a day at 9 am and 4 pm, and water was accessible *ad libitum*. Sampling was done every 2 weeks for a period of 12 weeks from the start of the Bt11 feeding. Samples of peripheral blood and rumen juice were collected 2 hr after feeding. This experiment was carried out in accordance with the guidelines for animal experiments of the National Institute of Animal Health (Tsukuba, Japan).

The hematological and biochemical parameters evaluated were as follows. Red blood cells (RBC), white blood cells (WBC), hematocrit (Ht), and hemoglobin (Hb) were measured with a Celltac MEK-5158 (Nihon-kohden, Tokyo, Japan). Aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), total-Bilirubin (T-Bil), total protein (TP), albumin (Alb), total cholesterol (T-Ch), triacylglycerol (TG), blood urea nitrogen (BUN), creatinine (Cre), calcium (Ca), inorganic phosphorus (iP), magnesium (Mg), and glucose (Glu) were determined on an Automatic Analyzer 7050 (Hitachi, Tokyo, Japan). Na, K, and Cl in serum were measured with an Electrolyte Analyzer IS-50C (Jokoh, Tokyo, Japan).

Samples of ruminal fluid were collected from calves via fistulae at two hours after feeding. The samples were squeezed through 2 layers of sterile gauze, and ruminal pH was measured using a pH meter. A part of the filtrate was centrifuged for 30 min at 30,000 g. The supernatant was filtered through a 0.2 μm filter, and was frozen at –20°C until the analyses. For volatile fatty acids (VFA) and lactic acid analyses, 1 m*l* of the preserved filtrate was mixed with 50 m*l* of 20% sulfosalicylic acid and centrifuged at 18,000 g for 10 min. The obtained supernatant was then applied to a high-performance liquid chromatography column (Shodex Ionpak KC-811, Showa Denko, Japan) and eluted with 3 mM $HClO_4$ at a flow rate of 1 m*l*/min. Eluates were visualized by post-column mixing with 0.2 mM bromothymol blue in 15 mM Na_2HPO_4 at a flow rate of 1.5 m*l*/min and detected by the absorbance at 445 nm. The concentration of ammonia nitrogen in the preserved filtrate was determined by Drichem 5500 (Fujifilm, Tokyo, Japan). The lipopolysaccharide (LPS) concentration in the preserved filtrate was measured by an automated turbidimetric-kinetic assay kit (Limulus ES-II Test Wako, Wako, Japan) as described previously [8].

After measurement, all values were expressed as a mean ± S.D. We evaluated the significance of the difference between mean values of Bt and control groups at each sampling week by Student's *t*-test. A level of $p<0.01$ was regarded as significant.

In this experiment, there was no effect of Bt11 corn feeding on body weight gain, body temperature, or gross clinical symptoms. The daily body weight gain of Bt11-fed and control calves was 0.99 ± 0.18 and 0.96 ± 0.17 kg/day, respectively. Calves in both groups showed no abnormal clinical signs such as fever, cough or diarrhea during the experiment.

All the hematological and biochemical parameters measured, i.e. RBC, WBC, Ht, Hb, AST, GGT, ALP, T-Bil, TP, Alb, T-Ch, TG, BUN, Cre, Ca, iP, Mg, Glu, Na, K, and Cl remained normal throughout the observation period in both groups. In Table 1 we present the values of those parameters at week 0 (just before the feeding experiment) and week 12 (the last week of the experiment). We also confirmed no abnormalities at any other in between sampling points. During the experiment, RBC gradually decreased and BUN increased in both groups. These shifts, however, might have reflected physiological adaptations correlating to the growth. Although two Bt11-fed calves and one control calf at week 0 had high AST activity (99 to 112 IU/*l*), these activities returned to normal levels after week 2. Therefore, we consider that the high AST activity of the three calves at the beginning of the experiment represented damage of hepatocytes for some reasons but was unrelated to the introduction of Bt11 feeding. It was clear that the continuous ingestion of Bt11 corn afterward did not affect those animals' liver function. The finding corresponds well with our recent *in vitro* knowledge that purified Cry1Ab, the key toxin expressed in the corn, does not damage the hepatocyte [11].

Similarly, there was no particular effect of Bt11 corn feeding on ruminal pH, VFA, lactic acid, ammonia nitrogen, or free LPS (Table 2). Although we only show the ruminal data at weeks 0 and 12, we confirmed no abnormalities at any other in-between sampling points. Both Bt11-fed and control calves had relatively high LPS and slightly low

Table 1. Hematological and biochemical data for calves in 3-month Bt11 corn-feeding trial

	Week 0		Week 12	
	Control	Bt11	Control	Bt11
RBC (× $10^4/\mu l$)	1010 ± 233	1022 ± 171	860 ± 66	873 ± 34
WBC (× $10^2/\mu l$)	91 ± 27	97 ± 26	102 ± 14	109 ± 24
Ht (%)	35 ± 3	37 ± 4	34 ± 1	34 ± 1
Hb (g/d*l*)	11.9 ± 1.6	11.8 ± 1.6	11.2 ± 0.3	11.3 ± 0.6
AST (IU/*l*)	57.5 ± 27.0	67.1 ± 27.4	43.5 ± 6.2	45.3 ± 5.3
GGT (IU/*l*)	16.5 ± 5.0	19.1 ± 2.3	16.1 ± 5.5	14.5 ± 1.5
ALP (IU/*l*)	328.5 ± 101.8	382.0 ± 115.0	427.6 ± 81.5	346.3 ± 61.4
T-Bil (mg/d*l*)	0.08 ± 0.03	0.09 ± 0.04	0.08 ± 0.05	0.08 ± 0.06
TP (g/d*l*)	6.4 ± 0.3	6.4 ± 0.3	6.6 ± 0.2	6.5 ± 0.1
Alb (g/d*l*)	3.5 ± 0.4	3.4 ± 0.2	3.7 ± 0.1	3.7± 0.1
T-Ch (mg/d*l*)	83.0 ± 17.8	70.8 ± 23.3	109.6 ± 20.7	93.0 ± 21.3
TG (mg/d*l*)	19.8 ± 6.1	14.5 ± 5.3	20.0 ± 9.2	14.8 ± 6.0
BUN (mg/d*l*)	11.1 ± 4.9	11.3 ± 5.2	15.9 ± 2.0	14.6 ± 1.3
Cre (mg/d*l*)	1.0 ± 0.1	1.0 ± 0.2	0.9 ± 0.1	1.0 ± 0.1
Ca (mg/d*l*)	10.1 ± 0.3	10.3 ± 0.3	10.2 ± 0.3	10.3 ± 0.3
iP (mg/d*l*)	8.5 ± 1.0	8.2 ± 1.1	9.4 ± 0.4	9.8 ± 0.5
Mg (mg/d*l*)	2.3 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	2.2 ± 0.1
Glu (mg/d*l*)	89.6 ± 12.5	86.3 ± 7.3	94.0 ± 6.9	93.0 ± 5.9
Na (mEq/*l*)	138.8 ± 1.9	137.1 ± 3.6	139.0 ± 2.4	138.8 ± 3.0
K (mEq/*l*)	4.4 ± 0.3	4.0 ± 0.1	4.1 ± 0.2	4.1 ± 0.2
Cl (mEq/*l*)	101.1 ± 1.1	98.5 ± 3.4	101.0 ± 1.9	100.5 ± 3.0

Values are expressed as means ± S.D. n=6.

Table 2. Ruminal data for calves in 3-month Bt11 corn-feeding trial

	Week 0		Week 12	
	Control	Bt11	Control	Bt11
pH	6.3 ± 0.3	6.1 ± 0.5	6.6 ± 0.1	6.3 ± 0.1
Lactic acid (mmoles/*l*)	ND	1.16 ± 2.59	0.04 ± 0.11	0.07 ± 0.18
Acetic acid (mmoles/*l*)	61.0 ± 12.1	60.2 ± 3.4	53.9 ± 12.9	53.3 ± 11.7
Propionoic acid (mmoles/*l*)	17.6 ± 5.1	22.7 ± 5.6	15.3 ± 4.9	17.0 ± 4.6
Butyric acid (mmoles/*l*)	13.8 ± 6.2	12.8 ± 5.2	10.5 ± 3.4	11.2 ± 3.3
Ammonia nitrogen (mg/d*l*)	16.7 ± 8.8	10.2 ± 9.0	16.1 ± 5.6	13.8 ± 6.8
LPS (μg/m*l*)	342.1 ± 204.9	1017.1 ± 798.3	460.3 ± 493.4	482.8 ± 543.2

Vales are expressed as means ± S.D. n=6. ND: Not detected.

ruminal pH, but no statistically significant differences were observed between the 2 groups, indicating that these changes were unrelated to the Bt11 feeding. We consider that this phenomenon was due to the amounts of concentrated feed given to calves, since both groups were given the high percentage of corn that theoretically maintained a balanced cattle diet.

In conclusion, the present results suggest that the insect-resistant transgenic corn Bt11 had no negative effects on clinical symptom, growth rate, hematology, blood biochemistry, or rumen functions of calves. It seems unlikely that Bt11 and its byproducts are harmful when fed to cattle.

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