The Fate of Genetically Modified Protein from Roundup Ready Soybeans in Laying Hens[1]

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Primary Audience: Researchers

SUMMARY

A study was conducted to determine the extent of genetically modified (GM) protein from Roundup Ready Soybeans in tissues and eggs of laying hens. Because a breakdown of the modified portion of protein was expected due to the digestive process of the hen, an immunoassay test was run. By using a double antibody sandwich format specific for the CP4 EPSPS protein, a qualitative test was performed to determine the presence of modified proteins in various samples. Raw soybeans, soybean meal, complete diet, whole egg, egg albumen, liver, and feces from laying hens were collected from two independent commercial egg producers. Roundup Ready soybeans, soybean meal, and complete diets were determined to contain the GM proteins. Whole egg, egg albumen, liver, and feces were all negative for GM protein. In conclusion, the digestive process of the laying hen effectively broke down the GM protein from the soybean meal portion of the diet, hence no modified protein was found in the liver, egg, or feces in this brief field trial.

Key words: genetically modified protein, Roundup Ready, soybean, egg, laying hen

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DESCRIPTION OF PROBLEM

In 1998, the U.S. produced 36.8 million tons of soybean meal, 50% of this meal was derived from genetically modified (GM) organisms [1]. Of that 36.8 million tons, 46% went into poultry production [1]. With only 8% of the Midwest grain elevators segregating non-GM soybeans from commodity soybeans [2], substantial portions of U.S.-produced poultry and poultry products are being generated using GM-derived soybeans.

Although the bulk of our poultry products do not go to export, over 4 million metric tons of poultry products were exported in 2001 [3], with a U.S. value of over $2 billion. This value is large enough to provide incentive for U.S. poultry producers to generate data that will address foreign consumer concerns over GM food safety issues with regard to poultry products.

Genetically modified products are currently at the forefront of agri-political debate, primarily as a result of consumer-based concerns over food safety issues such as consumption of GM organism (GMO) materials. Acceptance of transgenic

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technology is, however, becoming imperative as it has been reported that food production must be doubled by the year 2025 and almost tripled by the year 2050 [4]. In efforts to meet this escalating demand, agronomists are using transgenic technologies to design improved varieties of commercial grains and oilseed plants that manifest many beneficial traits. Already, improvements have been developed allowing crops to exhibit enhanced nutritional value, drought resistance and herbicidal tolerance [5].

With this still fairly new technology, GM variety crops have only been planted in the U.S. since 1996, but they have been well received by farmers. In the 1999 growing season, 50% of the U.S. soybeans and cotton and 30% of the corn were planted with genetically modified organisms [6]. Despite the widespread acceptance and incorporation of GM plant production into U.S. farming practices, consumer-based scrutiny abroad has compelled foreign markets to place labeling and import restrictions on GM products. A subsequent concern is that foreign markets will begin to scrutinize U.S. poultry meat and egg product exports produced from diets containing soybean meal from GM soybeans.

The objective of this study was to test various fractions of the egg, tissues, and feces from laying hens fed soybean meal produced from GM soybeans for any residual traces of the modified portion of Roundup Ready soybeans.

MATERIALS AND METHODS

Field samples were collected from two commercial egg producers. Samples collected from each location included whole soybeans (Roundup Ready), soybean meal, and complete layer diets and whole eggs, egg albumen, liver, and feces from two flocks at each location.

An immunoassay test [7] was used in this study. Antibodies specific to the CP4 EPSPS protein, the modified portion, were coupled to a color reagent and incorporated into a lateral flow strip. The antibody sandwich that formed around the modified portion of the protein was used to indicate the presence of the identifying segment. The kit provided a buffer, microcentrifuge tubes, and lateral flow strips.

Extensive preliminary testing was performed to determine the concentration of protein and dilution of samples required to give accurate results down to 0.1% of the CP4 EPSPS protein. Herein various dilutions of the separate fractions were run to adjust for protein content and flowability. Samples were prepared and diluted for subsampling and testing by immunoassay as follows. Twenty-gram samples of whole soybeans, soybean meal, or complete feed were ground and diluted with 100 mL distilled H_2O in a 200-mL beaker. After being mixed, a 0.5-mL sample was transferred to a microcentrifuge tube and centrifuged. Three drops of buffer from the Strategic Diagnostics, Inc. (SDI) [7] immunoassay test kit was added to the microcentrifuge tube, and then the lateral flow strip was dipped into the supernatant for 10 min to allow reaction of the sample protein with the antibody sandwich. Whole-egg sample was prepared by homogenizing two whole eggs, and then 0.5 mL of whole egg was transferred to a microcentrifuge tube. Three drops of buffer were added to the tube prior to testing with the lateral flow strip.

A 10-min reaction time was allowed with sample to determine a positive or negative reaction. Egg albumen was freeze-dried to concentrate dry matter and protein. One gram of freeze-dried albumen was diluted with 5 mL of distilled H_2O and homogenized before 0.5 mL was removed to a microcentrifuge tube. Buffer was added to the tube after centrifugation, and the lateral flow strip then reacted with the sample for 10 min. Fresh liver samples were taken from each farm and kept separate for sample preparation. Subsamples were homogenized with 125 mL H_2O in a blender. An aliquot of 0.5 mL was transferred to a microcentrifuge tube and centrifuged. Buffer was added to the supernatant, and the lateral flow strip reacted with the sample. Dry manure samples were also tested by homogenizing 10 g dry manure with 75 mL H_2O. A 0.5-mL subsample was transferred to a microcentrifuge tube for reaction with antibodies in the lateral flow strip as previously described. Each sample was run in quad-duplicate for the immunoassay test. Results were deemed positive when three out of four replicate results of a sample tested positive. Results were deemed negative when zero out of four replicates were positive and inconclusive when one or two tests out of four replicates were positive.

The immunoassay test was conducted on each subsample in four replicates. The ELISA

TABLE 1. Immunoassay test results for the CP4 EPSPS protein from Roundup Ready soybeans in feed and poultry tissues[A]

	Company 1		Company 2	
	Rep 1[B]	Rep 2	Rep 1	Rep 2
Roundup Ready	Yes	Yes	Yes	Yes
Soybean meal	Yes	Yes	Yes	Yes
Complete diet	Yes	Yes	Yes	Inconclusive
Whole egg	No	No	No	No
Egg albumen	No	No	No	No
Liver	No	No	No	No
Feces	No	No	No	No

[A]For results to be considered positive, at least three or four replicate samples had to be indicated positive. Negative results were zero out of four positive and inconclusive if one or two out of four replicates were positive. Assay sensitivity was 0.1% for the CP4 EPSPS protein.
[B]Average of four replicates per sample.

were conducted at a second, independent laboratory [8] to verify in-house immunoassay results for soybeans, feed samples, and whole-egg samples (two from each location). The ELISA test was sensitive to the presence of GM Roundup Ready protein at 0.1% concentration.

FIELD REPORT

When an animal ingests a soluble protein fraction, the protein is usually broken down during the normal course of digestion. The protein is degraded into di- and tripeptides as well as free amino acids, which are absorbed in the upper gut [9]. During this degradation process, it is hypothesized that protein from the transgenic portion of DNA is destroyed along with other proteins. Hence, the antibody sandwich in the lateral flow strips would no longer be able to find the identifying sequence. The immunoassay test positively indicated that CP4 SPSPS (Roundup Ready gene) was present as expected in the whole soybeans, soybean meal, and in the complete layer diets (Table 1) collected in this study from both companies. Whole soybeans were tested as a positive control because the immunoassay test kit was intended for detection in whole soybeans. It was, however, somewhat more difficult to gain conclusive results with the complete diet because the genetic material of the soybean was present at a lower percentage of the total diet as was evidenced by inconclusive results for company 2.

In this study, within the whole egg, egg albumen, or liver samples, no GM factions were found (Table 1) in any replicate from either company as a result of protein degradation. In the fecal samples, again, no modified portions of GM material were found. This result might have been due to a dilution factor. Some protein is expected to escape intact or partially intact and pass through into the excreta. The percentage of excreted intact protein is small, however, and of that percentage, the modified portion is even smaller. Hence, though some modified portions may be excreted, we have shown that this amount does not exceed 0.1% GM. Further analysis of soybean meal, layer feed, and whole-egg samples by ELISA at a separate lab [8] for the Roundup Ready gene confirmed the results of the strip test and showed positive results for feed samples but negative results for egg samples.

In a digestive fate study in mice, commissioned by Monsanto [10], the modified portion of the protein in Roundup Ready soybeans was completely digested. One could expect similar digestive breakdown in chickens, resulting in no GM factions found in the whole egg, egg white, or liver. Results of the study reported herein support these findings.

These tests may not have been sensitive enough to indicate potential undigested GM portions found in feces. However, data obtained in the present study support the fact that if intact GM portions are passing through the digestive tract of the hen, they are at levels less than 0.1%. At levels this low, we feel that concern over the use of fecal materials for fertilization or feed supplementation should not be an issue.

CONCLUSIONS AND APPLICATIONS

1. Roundup Ready soybean GMO protein is detectable by ELISA tests in soybean meal and complete diets fed to layer chickens at concentrations above 0.1%.
2. No Roundup Ready soybean GM protein was detectable in whole egg, egg albumen, liver, or excreta tissue. These results were confirmed by two separate labs for egg protein.
3. The laying hen is adept at digesting soybean meal protein to a stage at which no detectable intact GMO material is absorbed or deposited in tissues such as liver and eggs.

REFERENCES AND NOTES

1. American Soybean Association, 1999. 1999 Soy Stats. United Soybean Board. Chesterfield, MO.

2. Muirhead, S., 1999. Attention on GM grains, oilseeds becomes issue of acceptance. Feedstuffs 71(Oct. 4):14–21.

3. Agricultural Baseline Projections: U.S. Livestock, 2002–2011. http://www.ers.usda.gov/briefing/baseline/uslstock.htm. Accessed Oct. 2002.

4. Vasil, I. K., 1998. Biotechnology and food security for the 21st century: A real-world perspective. Nat. Biotechnol. 16:399–400.

5. Henry, R. J., and J. A. Ronalds, 1994. Improvement of Cereal Quality by Genetic Engineering. Plenum Press, New York.

6. Schuff, S., 1999. GMO testing will be factor in overseas grain, oilseed sales. Feedstuffs 71(Oct. 4):15–18.

7. Strategic Diagnostics, Inc., 1999. Trait Y GR Lateral Flow Test User Guide. Trait Y GR Bulk Soybean Test Kit. Strategic Diagnostics, Inc., Newark, DE.

8. Central-Hanse Analytical Lab., Belle Chasse, LA.

9. Scott, M. L., M. C. Nesheim, and R. J. Young, 1982. Nutrition of the Chicken. 3rd ed. M. L. Scott & Associates, Ithaca, New York.

10. Harrison, L. A., M. R. Bailley, M. W. Naylor, J. E. Ream, B. G. Hammond, D. L. Nida, B. L. Burnett, T. E. Nickson, T. A. Mitsky, M. L. Taylor, R. L. Furchs, and S. R. Padgette, 1996. The expressed protein in glyphosate-tolerant soybeans, 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp., Strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. J. Nutr. 126:728–740.