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FEEDING GENETICALLY ENHANCED SOY TO ANIMALS

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Abstract

Soybeans with transgenic events were approved for commercial use in the USA in 1996. Currently, the majority of soy produced in the US, Canada and Argentina is glyphosate resistant. Glyphosate or N-phosphono-methyl-glycine is the chemical name for Roundup® Herbicide. This is the only transgenic soy that has been approved for commercial use. To produce soybeans with tolerance to glyphosate, the EPSPS gene from the *Agrobacterium sp.* strain CP4 was cloned into a bacterial plasmid along with several other genes and DNA promoters to activate the genes. Copies of this plasmid were then delivered to soybean cells to produce a transgenic soybean or genetically enhanced soybean. Nutrients and antinutritional factors were evaluated in glyphosate tolerant soybean seed together with soybean meal derived from this seed. Proximate composition, urease activity, trypsin inhibitor and amino acids content of soybean and soybean meal obtained from glyphosate tolerance line is not significant different with the control soybean or soybean meal. There is also no difference in allergenicity between non-transgenic and glyphosate resistant soy. In vitro digestibility result indicated that the protein is digested by digestive enzymes. Feeding studies and laboratory analysis have been done to evaluate whether any differences exist between non-transgenic soy and glyphosate resistant soy. Studies in broilers, dairy cattle, rate and catfish have found no differences in nutritional value between non-transgenic and glyphosate resistant soy. New types of transgenic soy are currently being tested in the laboratory and hold promise for additional beneficial traits such as higher phosphate and nitrogen digestibility and higher levels of essential amino acids.

Introduction

Agricultural crops used as feed ingredients can be enhanced to improve yield or simplify agronomic practices, widen normal climatic conditions for growth or improve feeding value. Crops have been genetically enhanced for centuries by application of traditional breeding methods such as identification of pedigree traits, cross fertilization, backcrossing, hybridization and other methods. Most of these methods are very time consuming, taking years to decades or longer for improvements to be commercialized.

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In recent years since the identification of DNA and its structure and function by Watson and Crick in the 1960's, work has begun to identify genetic traits on the molecular level. It is now known that the structure of DNA contained in every living cell contains the genetic code for all the proteins and traits of the entire living organism. These include the many specific enzymes used to build the structure of the organism with biochemical and mineral components and also enzymes that regulate metabolic function. Since the early 1980's it has been possible to substitute fragments of DNA and change the metabolic expression of bacteria. More recently, these techniques have been applied to higher plants. In 1996, soybeans were commercialized with substituted fragments of DNA giving this crop a value enhanced trait. In a very short time the enormous potential both financial and as a way to provide more food and feed was widely recognized. At the same time, the technology has become increasingly controversial sparking debates on scientific ethics, morality and international trade. Since genetically enhanced soy reviewed and/or approved by the US Food and Drug Administration, US Department of Agriculture, Environmental Protection Agency, Animal and Plant Health Inspection Service, National Academy of Science in 1996 and similar organizations in South America, Europe and Asia, few people in the world have not consumed food products derived from this product. Soy derivatives such as oil, lecithin, protein concentrates and isolates, soybean meal, fiber, vitamin E and others are found in or required to produce many food products. These include meat (soybean meal in animal feed), candy and chocolate (soy lecithin and oil), fried foods (soy oil), bread (soy fiber), tofu (whole soybeans), soy sauce (soybean meal), noodles and processed foods (various soy proteins) soymilk, tempe and many other products.

Soybeans with transgenic events were approved for commercial use in the USA in 1996. Transgenic soy that is resistant to the environmentally friendly herbicide glyphosate contains less weed seeds, and has less possibility of being contaminated by mycotoxins or stabile herbicide residues. Farmers can control weeds more efficiently requiring less input of time and chemicals to the fields. Soon after 1996, many other countries including Canada, EU, Japan, Brazil and Argentina approved glyphosate resistant soy as safe for human and animal consumption. Currently, the majority of soy produced in the US, Canada and Argentina is glyphosate resistant. This is the only transgenic soy that has been approved for commercial use.

The purpose of this paper is to provide nutritionists and feed industry representatives with information on this new technology of genetic modification with focus on herbicide (glyphosate) tolerant soy. Consideration of issues and points of controversy are important to all people working in the food supply chain. It is imperative that all must be knowledgeable on this subject as it is the continued responsibility of this industry to supply safe, wholesome foods and keep the consuming public satisfied with what they eat.

Glyphosate Tolerant Soy

To date, the only commercially approved genetically enhanced transgenic soy in use today is glyphosate tolerant soy. This product has gained rapid acceptance by farmers since its commercialization in 1996 (Table 1) and was planted on 14 million hectares of land in the U.S. in 1999. This accounted for about 55% of total tonnage of beans in the U.S. and about 60% in Argentina. To date, no transgenic herbicide tolerant soy has been officially planted in Brazil or India although Brazil has approved safety of the crop.

Glyphosate or N-phosphono-methyl-glycine is the chemical name for Roundup® Herbicide. The structure as compared to glycine is given in Figure 1. Chemically is the carbon backbone of glycine with a methyl phosphonic acid side chain. Glyphosate is a non-selective herbicide. Glyphosate is a very safe water soluble herbicide with extremely low toxicity to animals and a very short half-life in the environment. Glyphosate is rapidly broken down to CO_2 and inorganic phosphate by soil microbes. Its herbicidal properties are due to the ability of the molecule to bind with and inactivate the enzyme EPSPS or 5-enolpyruvate shikimate-3-phosphate-synthase contained in plants (Steinrucken and Aurheim, 1980). This is a required enzyme of the shikimic acid pathway responsible for the biosynthesis of aromatic amino acids. Specifically, EPSPS catalyzes the reaction of shikimate-3-phosphate and phosphoenolpyruvate to form 5-enolpyruvyl shikimate-3-phosphate and phosphate. The enzyme EPSPS is necessary for the production of tyrosine, tryptophan and other aromatic amino acids and secondary metabolites. Inhibition of EPSPS by the herbicide glyphosate thus prevents plants from synthesizing protein. The enzyme EPSPS is present in all plants, bacteria and fungi but not in animals. This is why animals are unaffected but also has a requirement for aromatic amino acids in the diet. Glyphosate has been demonstrated to have low binding to the EPSPS enzyme contained in many species of bacteria.

The naturally occurring EPSPS enzyme from the bacteria *Agrobacterium sp.* strain CP4 was identified from a screen of microorganism cell extracts as being glyphosate tolerant. In the presence of glyphosate, the EPSPS derived from *Agrobacterium sp.* strain CP4 was still able to produce the 5-enolshikimate-3-phosphate necessary for aromatic amino acid synthesis (Padgette *et al.*, 1995). To produce soybeans with tolerance to glyphosate, the EPSPS gene from the *Agrobacterium sp.* strain CP4 was cloned into a bacterial plasmid along with several other genes and DNA promoters to activate the genes. Copies of this plasmid were then delivered to soybean cells using a particle acceleration transformation system or "gene gun". In this technique, the cloned DNA is mixed with inert pellets (usually gold particles) and literally blasted into the soybean cells using a gunpowder charge. Soybean cells are then separated, grown into small plantlets in tissue culture and tested for presence of cloned DNA. Plant tissue showing the new trait are then grown to full size and further tested in greenhouses. Seeds are collected and progeny grown in larger scale field evaluations. Further multiplication steps are then necessary to produce enough seed for commercialization. The gene for glyphosate

tolerance has been determined to be dominant and persists over multiple generations. Because commercial soybean varieties are not hybrids, seed collected from plots of genetically modified soy can be saved for growth in subsequent years.

Glyphosate resistant soy contains a gene from one or more bacteria such as *Agrobacterium tumefactions*. This bacterial gene codes for production of an enzyme, EPSPS (5-enol-pyruvyl shikimate-3-phosphatesynthase) which is resistant to binding by glyphosate herbicide. The bacterial EPSPS has a slightly different amino acid sequence compared to the normal soy EPSPS. This difference is enough to reduce its binding ability to glyphosate. The enzyme EPSPS is important in production of tryptophan in plants. When bound by glyphosate the plant cannot produce tryptophan and dies. Plants containing the bacterial enzymes are unaffected by the herbicide glyphosate.

Antibiotic Resistant Markers

Genes that code for enzymes that inactivate antibiotics have been used as selectable markers in the development of glyphosate tolerant soy and other transgenic species. Plasmid or circular strands of bacterial DNA are used to construct gene sequences containing the CP4-EPSPS gene, antibiotic resistant marker gene nptII and some gene promoters. The nptII gene codes for expression of the enzyme neomycin phosphotransferase which inactivates the antibiotics neomycin and kanamycin. The presence of nptII ensures that the new plasmid contains the CP4-EPSPS gene. After introduction of the new DNA into the plant cells, kanamycin is applied to kill untransformed tissue. Transformed cells expressing the neomycin phosphotransferase enzyme are protected from the effects of the antibiotic and using appropriate culture media can be regenerated into whole transgenic plants (Huppatz, 1998). The transgenic plant cells do not contain antibiotics and they do not produce antibiotics. Genetically modified plants have no effect on antibiotic resistance of bacteria in the soil or in the gastrointestinal tract. Any antibiotic therapy the animal is being treated with is not affected by glyphosate tolerance soy or any other transgenic crop. DNA produced by soybeans whether natural or transgenic is fragmented during meal processing and digestion. Thus the possibility for a gene-sized fragment of DNA getting into the gut is very small. Following ingestion, DNA digestion begins within the oral cavaty by enzymatic catalysis with DNAase I which is secreted by the salivary glands and has optimal activity at neutral pH (Beever, 1998). The low pH of the stomach further hydrolyzes DNA. Furthermore there is no evidence that transfer of plant DNA to gut microflora can occur. This has been examined under a wide variety of conditions by several independent research teams with negative results (Huppatz, 1998). However if this most unlikely chain of event did occur, and the bacteria somehow became resistant to the antibiotic kanamycin, there would be no effect on animals or humans as this antibiotic is not used in medicine. The majority of genetically modified crops species containing antibiotic resistant marker genes have been evaluated and

approved by various government agencies around the world. However responding to public concern, laboratories developing new varieties of genetically modified crops are now using alternative markers such as herbicide resistant genes.

Recently, the effects of soybean processing on DNA fragmentation have been examined (Smith *et al.*, 2000). DNA was extracted from soybeans, final processed soybean meal and in samples taken at various points along the processing chain. The presence of functional gene sized fragments of 500 base pairs or longer was evaluated. In whole fresh soybeans, extraction of 23,000 base pair fragments has been demonstrated. Results in processed soy were variable with some samples showing no DNA fragments and others showing fragments greater than 500 base pairs. Different sources of the soybean meal produced different results suggesting that processing sometimes but not always destroys DNA by fragmentation. In contrast, the evaluation failed to find DNA in every sample of corn gluten meal and rapeseed meal was failed to detect any gene size fragments in any sample of corn gluten meal or rapeseed meal, likely reflecting the higher processing temperatures used to produce these commodities as compared to soybean meal.

Allergenicity Assessment

Expression of unexpected allergenic protein from genetically modified plants has been a concern of critics of this technology. Non-GMO soy protein is naturally allergenic as are proteins contained in other foods such as fish, crustaceans, peanuts, mollusks, eggs, nuts, milk and wheat. Baby mammals and some adult humans are sensitive to these. Early transgenic experiments to develop a high methionine soybean by inserting a gene to express Brazil nut protein were discontinued due to the allergenicity of the Brazil nut protein.

Before approval for commercial use, allergenicity of protein from glyphosate tolerant soy was examined by two methods. First, the endogenous protein and levels present in commercially available non-GMO soybean were compared to those present in soybeans containing the CP4- EPSPS gene and expressed enzyme. No differences were found in the composition or relative quantities of allergens (Burkes and Fuchs, 1995). Second, the biochemical properties of known allergenic protein were compared to CP4-EPSPS. Except for its size of 47,600 Daltons (about 255 amino acids) CP4-EPSPS possesses none of the other common characteristics common to protein allergens. CP4-EPSPS is not heat stable and all detectable functional activity is lost after processing into soybean meal (Duke, 1996). CP4-EPSPS from raw soybeans was found to be extremely labile to digestive enzymes. The enzyme EPSPS and CP4-EPSPS are not glycosylated proteins, unlike most allergenic proteins. CP4-EPSPS is functionally similar to soybean EPSPS and shows no amino acid sequence homology to any known protein allergen. CP4-EPSPS and endogenous soybean EPSPS show 51% similarity in amino acids and 26% similarity in amino acid sequence. Finally most allergens in food are present as major protein components. In contrast, CP4-EPSPS represents approximately 0.03% of the total

fresh weight of a soybean seed and 0.08% of the total protein content of the seed (Duke, 1996).

Chemical composition and digestibility

Soybean

Nutrients and antinutritional factors were evaluated in glyphosate tolerant soybean seed over two growing years and compared to similar parental non-GMO lines (Padgette *et al.*, 1996). No differences in protein, ash, moisture, fat, fiber or carbohydrate content were found. The soybean meals used in the animal feeding experiments was also examined and found to be equivalent in protein, ash, moisture, fat, fiber, carbohydrates, phytate, stachyose, raffinose, urease, trypsin inhibitor, nitrogen solubility, and lectin content. The isoflavones genestein, daizdein, coumesterol and biochanin A were also measure and found to be very similar between the meals and all within published literature values for soybean meal. The amino acid levels and profile of beans and meal from glyphosate tolerant and non-glyphosate tolerant parental lines were found to be substantially equivalent (Table 2.). The lack of alteration of aromatic amino acid levels in GTS soy was in accordance with the expectation that EPSPS was not the rate limiting step in aromatic amino acid synthesis in bacteria and plants.

Soybean meal

Proximate composition, urease activity and trypsin inhibitor content of soybean meal obtained from gluphosate tolerance line is presented in Table 3. There is no significant different between the new line with the control soybean meal obtained from existing commercial soybean meal. The Table indicated that there is no significant different in trypsin inhibitor content and urease activity, used as marker enzyme to measure the extend of processing. Similarly, the level of amino acids measured from 18 different amino acids both for essential and not essential for monogastric animals were are not different. Soybean meal contains a very high in lysine, tryptophan and threonine but limited in methionine (Hammond et al., 1996).

An assessment of digestion by enzymatic activity had been performed by an in vitro technique using enzyme pepsin and pancreatin. CP4 EPSPS protein isolated from the new line during was incubated with the digestive enzymes and the protein was measured by Western blots technique. The results indicated that there was no activity of CP4 EPSPS after 2 minutes incubation (Table 4). In 2 experiments performed, CP4 EPSPS activity was decreased to <10 % after less than 5 hr incubation. This indicates that CP4 EPSPS protein was digested well in the digestive system of monogastric animals (Harrison et al., 1996).

Feeding Value of Glyphosate Tolerant Soy

The feeding value of soybeans was not found to be altered by genetic incorporation of glyphosate tolerance (Hammond *et al.*, 1997). Rats, chickens, catfish and dairy cattle were fed similar genetic lines of soybeans, two with incorporated glyphosate tolerance lines (40-3-2 or GTS A and 61-67-1 or GTS B) and the parental non-GMO commercial line A5403. The beans were grown in test plots, harvested and processed into soybean meal at the pilot crushing plant of the Food Protein Research Center at Texas A& M University and incorporated into diets and fed to animals.

In the broiler study, separate sex feeding of the three lines of meal was conducted with diets formulated to contain approximately equal amounts of six dietary essential amino acids (methionine, cystine, lysine, arginine, tryptophan and threonine). Diets contained no medications of feed additive growth promotants or know contaminants. The starter diet was pelleted and crumbled and fed for the first 21 days. The grower diet was pelleted and fed from day 21-42. Table 5 shows the diet composition.

For the cumulative 42 day study period (0-42 days) no differences in body weight, live weight, feed intake, feed conversion or livability were observed. Males, as expected were heavier, consumed more feed and had more efficient feed conversion than females (Table 6). There were no differences among soybean meal groups in breast muscle or abdominal fat weight. There were no soybean meals by sex interactions. As expected, males had more breast muscle and less abdominal fat than females.

In the other studies reported using catfish, rats or dairy cattle, no performance differences were found in animals fed soybean meal made from commercial sources or that made from glyphosate tolerant soybeans.

Normally, animal feeding studies are not used to evaluate the quality of new varieties of soybeans that are generated in commercial plant breeding programs. Compositional analysis of the new soybean varieties is considered sufficiently sensitive to assess nutritional quality and the practice has worked well through the years. The results of these studies indicate that compositional chemical analysis is in fact adequate to detect potential material differences in new genetically modified lines.

Laboratory Detection of Glyphosate-Tolerant Soy

Two methods can be used to detect the presence of the CP4-EPSPS gene and or its expressed CP4-EPSPS protein in glyphosate tolerant soy. Comparative details are given in Table 7. The enzyme linked immunosorbent assay or ELISA method can be used to detect CP4-EPSPS protein and relies on specific interactions between antibodies and antigens to measure substances. The key reagents are antibodies produced in the immune system of a live animal to a specific protein namely CP4-

EPSPS. When the antigen CP4-EPSPS binds to the specific antibody it can be visualized by a colorimetric of fluormetric reaction. ELISA is reliable for intact protein such as in soybean seed or plant tissue. It is not reliable for processed proteins and would be expected to give a high number of false negatives.

The polymerase chain reaction or PCR method detects presence of a specific sequence of DNA. In this test, very small amounts of DNA are amplified to produce millions of copies of a specific base pair sequence in a few hours. The method involves extraction of DNA, amplification of DNA and electrophoretic analysis of the PCR products. While sensitive enough to be used in forensic investigations, this test must have intact fragments of DNA present long enough to preserve a sequence unique to the organism being detected. At time if this writing there have been few if any reports on the accuracy of this test to detect soybean meal made from glyphosate tolerant soybeans. Based on recent work on the effect of processing on the fragmentation of DNA in animal feed ingredients (Smith *et al.*, 2000), the PCR test like the ELISA test would be expected to give false negative results or in other words not be able to detect all soybean meal made from glyphosate tolerant soy.

Conclusion

Genetic improvements through traditional breeding and DNA modification are increasing at an rapid rate. At present, glyphosate tolerant soy has received approval for commercial planting in the U.S., Canada and Argentina and is gaining wide acceptance by farmers. The gene for glyphosate tolerance can be applied to a wide range of soy cultivars. This characteristic allows the farmer to use the broad spectrum and environmentally friendly herbicide, glyphosate, during virtually any stage of growth. Yield improvements, lower herbicide application and significantly lower cultivation costs are gained. The finished product is nutritionally the same as other soy with lower probability of weed seed contamination and pesticide residue. Preservation of identity is not necessary for farmers to gain benefit with glyphosate tolerant soy but is required for markets where labelling of such products or their derivatives is required by the public.

Other genetic improvements being tested now differ from glyphosate tolerance in that they improve nutritional value. Recent alliances and acquisitions between companies, Governments and universities will push the development of identity preserved soy with value-added nutritional traits. Traits such as high lysine, high methionine, phytase enzyme activity, altered fatty acid composition, and decreased trypsin inhibitor are on the horizon as suggested in Table 8. Feed industry representatives should endeavor to keep up with the pace of this new technology to gain a full understanding of its potential benefits and risks. Sooner or later all members of the feed industry will be required to discuss this as an authority on the subject.

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H_2O_3-P-CH_2-NH-CH_2-COOH
(Glyphosate (N-phoshono-methyl-glycine)

H_3N-CH_2-COOH
(Glycine)

Figure 1.Chemical Structure of Glyphosate

Table 1.Plantings of Genetically Modified Soy in the U.S.

Year	Percent
1996	2
1997	12
1998	40
1999	55
2000	50

Table 2. Amino acids composition of soybean seed from Glyphosate Tolerance line (%DM).

	A5403 (control)	GTS 40-3-2[2]	GTS 61-67-1[2]
Aspartic acid	4.53	4.42	4.48
Threonine	1.60	1.56	1.58
Serine	2.10	2.04	2.07
Glutamic acid	7.34	7.10	7.26
Proline	2.03	1.98	2.02
Glycine	1.72	1.67	1.69
Alanine	1.71	1.67	1.69
Valine	1.85	1.80	1.83
Isoleucine	1.78	1.73	1.76
Leucine	3.05	2.97	3.03
Tyrosine	1.45	1.40	1.43
Phenylalanine	1.97	1.90	1.95
Histidine	1.06	1.03	1.04
Lysine	2.61	2.56	2.58
Arginine	2.94	2.85	2.90
Cysteine	0.60	0.62	0.60
Methionine	0.55	0.55	0.54
Tryptophan	0.59	0.59	0.58

From: Padgette et al. (1996)

Table 3. Proximate composition, urease activity and trypsin inhibitor content of deffated untoasted of Glyphosate Tolerance soybean meal.

Component	Seed line		
	A5403 (control)	40-3-2	61-67-1
		g/100 g dry wt (unless noted)	
Protein	53.2	53.6	52.8
Ash	6.53	6.89	6.65
Moisture, *g/100 g fresh wt*	6.55	11.90	4.17
Fat	2.30	0.73	2.13
Fiber	4.52	4.23	3.71
Carbohydrates	38.0	38.8	38.4
Ureases, *pH*	2.30	2.45	2.19
Trypsin inhib.,7			
TIU/mg dry wt	65.9	83.5	73.6

From Padgette et al. (1996)

Table 4. Dissipation of CP4 EPSPS enzymatic activity in simulated digestive enzyme.

	Experiment 1		Experiment 2
Incubation time	CP4 EPSPS activity[2]	Activity remaining	CP4 EPSPS activity[2]
min		%	
0	21.3	100	32.2
10	20.3	95	20.0
270		-	<2.0
285	<2.0	9	-

From Harrison et al. (1996)

Table 5.Glyphosate Tolerant Soy Broiler Feeding Study:Ingredient Composition and Proximate Analysis of Diets

	Starter diet			Grower diet		
	Commercial	GT Soy A	GT Soy B	Commercial	GT Soy A	GT Soy B
	g/100 g dry matter (unless noted)					
Corn	58.0	56.9	58.1	63.7	62.8	63.7
Soybean meal						
Commercial	32.8	-	-	26.6	-	-
GT Soy A	-	33.8	-	-	27.4	-
GT Soy B	-	-	32.9	-	-	26.6
Soybean oil	4.0	4.2	4.0	4.7	4.8	4.6
Methionine	1.5	1.5	1.4	1.6	1.6	1.5
Choline	0.003	-	0.003	-	-	-
L-Lysine	0.002	-	-	0.025	0.01	0.008
Limestone	1.4	1.4	1.4	1.3	1.3	1.3
Ca_2PO_4	1.6	1.6	1.6	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5	0.5	-
Vitamins[2]	0.1	0.1	0.1	0.1	0.1	0.1
Minerals[3]	0.1	0.1	0.1	0.1	0.1	0.1
Energy,	13.2	13.2	13.2	13.6	13.6	13.6
MJ(kcal)/kg	(3155)	(3155)	(3155)	(3250)	(3250)	(3250)
Crude protein	20.80	21.40	20.80	18.10	17.97	18.31

from: Hammond *et al.*, 1996

Commercial = non-GMO soy line A5403, GT Soy A = line 61-67-1, GT Soy B = line 40-3-2

Table 6.Glyphosate Tolerant Soy Broiler Feeding Study:Performance Results

	Soybean line			Sex	
	Commercial	GT Soy A	GT Soy B	Female	Male
Number	120	120	120	180	180
Body weight, g	2192	2188	2144	2041a	2309[b]
Daily gain, g/d	51	51	50	48[a]	54[b]
Daily feed consumed, g/d	93	93	92	88[a]	97[b]
Feed/Gain, g/g	1.815	1.825	1.832	1.848[a]	1.799[b]
Livability, %	90.8	89.2	91.7	93.9[d]	87.2[c]
Breast weight, g	302	296	294	284[a]	311[b]
Breast/body weight, g/100 g	13.8	13.5	13.7	13.9[b]	13.4[a]
Fat Pad weight, g	81	82	77	85[b]	75[a]
Fat Pad/body weight, g/100 g	3.7	3.8	3.6	4.2[a]	3.3[b]

a,b means with different superscripts and different (P<.0.5)
from: Hammond *et al.*, 1996

Table 7.Comparison of Genetically Modified Soy Detection Techniques

PCR and related techniques:	ELISA and related techniques:
detect DNA sequences on the basis of their uniqueness;	detect proteins on the basis of their specific interaction with antibodies;
no detection possible in the absence of DNA;	no detection possible in the absence of proteins;
are extremely sensitive;	are less sensitive but
require very careful experimental set-up as well as data interpretation to ensure reliability	are very reliable;
require the analysis of reference material;	require the analysis of reference material;
require standardization for sampling and extraction of material;	require standardization for sampling and extraction of material;
require detailed knowledge of the molecular structure of the introduced sequences;	require a detailed knowledge of the molecular structure and of the physico-chemical properties of the protein;
in most cases provide a qualitative yes/no answer (*);	provide a quantitative and qualitative answer;

From: Van den Eede *et al.* 1999

Table 8. Likely Commercialization of Value Added Soybean Varieties

Soybean Trait	Year	Company
Glyphosate tolerance	1996	Monsanto
High oleic oil	1998	Monsanto, Dupont,Pioneer
Low phytate	2001	Dupont
Glufosinate tolerant	2001	Agrevo
High lysine	2002	Dupont, Renessen
Low stachyose	2004	Dupont, Pioneer
Low lipoxygenase	2004	Monsanto, Dupont
High methionine	2005	Dow, Monsanto
Antibody containing	2007	Renessen, Monsanto, Dupont
Biodegradable plastic	2010	Monsanto, Dow