The Feeding Value of Soybeans Fed to Rats, Chickens, Catfish and Dairy Cattle Is Not Altered by Genetic Incorporation of Glyphosate Tolerance[1,2]

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ABSTRACT Animal feeding studies were conducted with rats, broiler chickens, catfish and dairy cows as part of a safety assessment program for a soybean variety genetically modified to tolerate in-season application of glyphosate. These studies were designed to compare the feeding value (wholesomeness) of two lines of glyphosate-tolerant soybeans (GTS) to the feeding value of the parental cultivar from which they were derived. Processed GTS meal was incorporated into the diets at the same concentrations as used commercially; dairy cows were fed 10 g/100 g cracked soybeans in the diet, a level that is on the high end of what is normally fed commercially. In a separate study, laboratory rats were fed 5 and 10 g unprocessed soybean meal 100 g diet. The study durations were 4 wk (rats and dairy cows), 6 wk (broilers) and 10 wk (catfish). Growth, feed conversion (rats, catfish, broilers), fillet composition (catfish), and breast muscle and fat pad weights (broilers) were compared for animals fed the parental and GTS lines. Milk production, milk composition, rumen fermentation and nitrogen digestability were also compared for dairy cows. In all studies, measured variables were similar for animals fed both GTS lines and the parental line, indicating that the feeding value of the two GTS lines is comparable to that of the parental line. These studies support detailed compositional analysis of the GTS seeds, which showed no meaningful differences between the parental and GTS lines in the concentrations of important nutrients and antinutrients. They also confirmed the results of other studies that demonstrated the safety of the introduced protein, a bacterial 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. strain CP4. J. Nutr. 126: 717–727, 1996.

INDEXING KEY WORDS:

• *glyphosate-tolerant soybeans* • *rats* • *catfish* • *chickens* • *dairy cows*

The two previous papers in this series (Harrison et al. 1996, Padgette et al. 1996) addressed the compositional equivalence and the safety of the protein expression product of soybeans that have been genetically modified to be tolerant to the herbicide glyphosate [glyphosate-tolerant soybeans (GTS)[4]]. A description of the procedures used to introduce the gene that imparts glyphosate tolerance into a commercial soybean cultivar has been published elsewhere (Padgette et al. 1995). The first paper in this journal series presented the results of extensive compositional analyses that demonstrated that GTS seeds are substantially equivalent to the commercial parental soybean variety (Padgette et al. 1996). The second paper in this series dealt with the safety of the protein expression product of the cloned gene, 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. strain CP4 (CP4 EPSPS), which is highly resistant to inhibition by glyphosate (Harrison et al. 1996). Glyphosate-tolerant soybeans expressing CP4 EPSPS are able to grow and develop normally after treatment with glyphosate.

Although the compositional studies confirmed the equivalence of GTS to commercial soybean varieties, animal feeding trials were undertaken to provide further support for commercial acceptance of this new soybean variety. Because soybeans are a major source of protein in the diets of most farm animals, as well as the diets

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[4] Glyphosate is the active ingredient of the broad-spectrum, nonselective herbicide Roundup®, and GTS are also denoted as Roundup Ready™ soybeans (Monsanto Company, St. Louis, MO).

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of laboratory and companion animals, four different species (laboratory rats, broiler chickens, catfish and dairy cattle) were fed diets containing processed or ground soybeans. The unmodified parental line was compared with two genetically modified GTS lines. Rats were fed either processed or ground soybeans separately in two trials, although ground soybeans are not normally fed to nonruminants due to the presence of antinutritional factors in the beans that are inactivated by processing (Leiner 1994). Because many dairy cattle are fed raw, unprocessed soybeans, we also fed raw soybeans to lactating dairy cattle. Catfish and broiler chickens were fed processed soybean meal, which is a normal component of their commercial diets.

MATERIALS AND METHODS

General. Two GTS lines (designated as 40-3-2 and 61-67-1) and a parental variety (a commercial soybean, A5403) were utilized in all animal feeding studies. Currently, only line 40-3-2 is being developed for commercial use. However, the results of the animal feeding studies with line 61-67-1 will be included in this publication to increase the power of the statistical tests to detect differences. Information on the sources and processing of the soybeans used is found in the first publication in this series (Padgette et al. 1996). All studies were conducted in general conformance with federal Good Laboratory Practice guidelines (U.S. Food and Drug Administration 1987). Animal housing and husbandry were in accordance with the *Guide to the Care and Use of Laboratory Animals* (NRC 1985). Glyphosate-tolerant and parental-line soybeans grown from the same field test sites were used in all of the feeding studies.

Rat study processed soybeans. Male and female Sprague Dawley (CD®) rats (Charles River, Portage, MI) were housed singly and given free access to water and rodent diet containing the processed parental line or GTS meal for approximately 1 mo (Monsanto Environmental Health Laboratory, St. Louis, MO). Rats were classified by weight and assigned to groups of 10 rats/sex so that all groups had equal initial body weights. Rats were approximately 8 wk of age at study start. Diet consumption and body weight were measured for each rat on a weekly basis. Rats were observed twice daily. In addition to the rats fed the parental line and two GTS lines, a fourth group (diet control) was fed nonpurified diet (Purina Laboratory Rodent Chow® 5001, Purina Mills, St. Louis, MO) that did not contain GTS or parental line processed soybeans. At the end of the study, all test animals were killed by carbon dioxide asphyxiation and necropsied. Liver, testes and kidneys were weighed, and approximately 40 tissues were collected for each animal and saved in formalin.

Soybeans from parental and GTS lines were grown at the same time and in the same field test plot [Marion, AR, in 1992; denoted as "large-scale study" in the first paper in this series (Padgette et al. 1996)]. Soybeans were processed (dehulled, defatted, toasted) into meal at the Food Protein Research and Development Center (Texas A&M University, College Station, TX) and shipped to Purina Test Diets (Richmond, IN) for formulation into rodent diets. On the basis of the proximate analysis of the meal, rodent diets were formulated to be isonitrogenous and similar to the nutrient profile for Purina Laboratory Rodent Chow 5001 diet (24.7 g/100 g protein, 4.5 g/100 g fat, 5.4 g/100 g fiber, 6.9 g/100 g ash). Processed soybean meal from each line was incorporated into the rodent diets at the same substitution levels used commercially (24.8 g/100 g).

After ANOVA, Dunnett's multiple comparison test (two-tailed) was used to detect significant differences ($P \leq 0.05$) between GTS-fed groups and their controls for body weight, cumulative weight gain and food consumption (Dunnett 1955). Terminal body weights and absolute and relative organ weights were evaluated for statistical differences ($P \leq 0.05$) by decision-tree statistical analysis procedures. Depending on the results for normality (Health Science Computing Facility 1977) and homogeneity of variance (Barlett's test), either parametric (Dunnett's test and linear regression) or nonparametric (Kruskal-Wallis, Jonckheere's and/or Mann-Whitney tests) routines were used to detect group differences and analyze for trend (Breslow 1970, Draper and Smith 1966, Hollander and Wolfe 1973, Mann and Whitney 1947, Snedecor and Cochran 1967). Other statistical routines used for some data were Barlett's test (Dixon and Massey 1969) to evaluate homogeneity of variances and Grubb's test (Grubbs 1969, Grubbs and Beck 1972) to detect for outliers.

Rat study (ground soybeans). Ground soybeans were included in the diet fed to rats for approximately 1 mo. Glyphosate-tolerant and parental line soybeans were obtained from the Puerto Rico field test discussed in the first paper in this series (Padgette et al. 1996). Soybeans were ground into fine powder but not further processed and then formulated into rodent diets at concentrations of 5 and 10 g/100 g. Feeding rats ground soybeans at concentrations greater than 10 g/100 g has been reported to produce growth retardation due to the presence of antinutritive factors (Kakade et al. 1973). The same procedures discussed before for formulation of test diets were followed except that the ground soybeans were added at a level of 5 or 10 g/100 g diet, replacing on an isonitrogenous basis only some of the processed soybean meal normally added to rodent diet formulated by Purina Mills. The experimental design for the rat feeding study with unprocessed soybean meal was the same as that for the rat study using processed soybeans. Because feeding rats unprocessed soybean meal has been reported to cause hypertrophy of the pancreas due to the presence of trypsin inhibitors (Leiner and Kakade 1980), the pancreata of all animals were examined microscopically.

TABLE 1

Ingredient composition and proximate analysis of diets for broiler chicken study

	Starter diet			Grower diet		
	A5403	GTS 61-67-1	GTS 40-3-2	A5403	GTS 61-67-1	GTS 40-3-2
	g/100 g dry matter (unless noted)					
Corn	58.0	56.9	58.1	63.7	62.8	63.7
Soybean meal						
Line A5403	32.8	—	—	26.6	—	—
Line GTS 61-67-1	—	33.8	—	—	27.4	—
Line GTS 40-3-2	—	—	32.9	—	—	26.6
Soybean oil	4.0	4.2	4.0	4.7	4.8	4.6
Alimet[1]	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, *MJ/kg*	13.2	13.2	13.2	13.6	13.6	13.6
Crude protein	20.80	21.40	20.80	18.10	17.97	18.31

[1] Alimet® feed supplement (Novus International, St. Louis, MO) is an 88% aqueous solution of 2-hydroxy-4-methylthiobutanoic acid.
[2] Vitamin premix provided the following (mg/kg diet): thiamin mononitrate, 1.1; riboflavin, 8.8; *d*-calcium pantothenate, 13.2; niacin, 38.5; pyridoxine, 3.3; folacin, 0.99; biotin, 0.055; vitamin B-12, 0.013, menadione dimethylpyrimidinol bisulfite, 2.64; ethoxyquin, 125; retinyl A acetate, 3.027; cholecalciferol, 0.082; all-*rac*-α-tocopherol, 12.
[3] Trace mineral premix provided the folowing (mg/kg diet): manganese, 64; zinc, 70; iron, 50; copper, 8; selenium, 0.3; iodine, 0.8.

The same statistical procedures outlined for the processed soybean rat feeding study were followed for analysis in this study. Because the pancreas was examined microscopically for all animals in this study, Fisher's exact test was used to compare the incidence of microscopic changes (Fisher 1946).

Poultry study. This study was conducted at the Dardenne Poultry Research Center (NOVUS International), Dardenne, MO. Three hundred sixty newly hatched Cobb 500 commercial broilers (Tyson Foods, Springdale, AR) were feather-sexed and placed by apparent sex into cages (five birds/cage). Cages were in three tiers in two battery rooms. Treatments were assigned randomly within cage tier so that each tier contained two cages per treatment with a 3 × 2 factorial arrangement of three diets and two sexes. The three test diets were formulated by NOVUS International (St. Louis, MO) based on amino acid analysis of the soybeans. All test diets were formulated to contain approximately equal amounts of six dietary essential amino acids (methionine, cystine, lysine, arginine, tryptophan and threonine). The diets contained no medications, feed-additive growth promotants or known contaminants. A broiler starter diet was fed from d 0 to 21 as crumbled pellets and from d 22 to 42 as pellets. Diet composition is presented in **Table 1.**

Weights and feed consumption were determined weekly during the 6-wk study. Weight gain, feed consumption and feed efficiency were calculated for each pen. Birds were observed daily, and any birds that escaped from cages were weighed and removed from the study. Dead birds were weighed and necropsied and the apparent cause of death determined. Pen feed efficiency was calculated by dividing grams of weight gained by live birds plus weight gained by dead and culled birds prior to removal by total grams of feed consumed. Livability was defined as the total number of live birds remaining in each pen at each weigh period as a percentage of the number of birds placed on d 0.

After the completion of the growth phase of the study, the major and minor pectoralis muscles from the right side and abdominal fat pads were dissected and weighed. Gonads were examined to verify the sex of each bird.

Data were analyzed by ANOVA for a 3 × 2 × 3 factorial arrangement of treatments (3 diets × 2 sexes × 3 cage tiers). Tier effects were nested within two rooms, and residual error mean square was used to test significance.

Catfish study. The Delta Research and Extension Center, Mississippi State University, performed the 10-wk catfish feeding study and formulated soybean meal into catfish diets based on proximate analysis of the meal. Processed soybean meal from each line was incorporated into the catfish diets isonitrogenously at the same substitution levels used commercially (45–47 g/100 g) (**Table 2**); the final concentration of protein in the catfish diets was 32 g/100 g.

Fingerling channel catfish (Ictalurus punctatus, Mississippi Select strain raised from eggs at the Delta Re-

TABLE 2

Ingredient composition of diets for catfish study[1]

	Soybean line		
	A5403	GTS line 61-67-1	GTS line 40-3-2
	g/100 g total diet		
Soybean meal	45.00	47.05	47.15
Dicalcium phosphate	1.00	1.00	1.00
Wheat midds	19.98	19.98	19.98
Catfish oil	1.50	1.50	1.50
Mineral mix[2]	0.10	0.10	0.10
Corn	22.18	20.13	20.03
Meat/blood-65	4.00	4.00	4.00
Fish meal, menhaden	4.00	4.00	4.00
Stay c®[3]	0.13	0.13	0.13
Ascorbate-free vitamin mix[4]	0.10	0.10	0.10
Pellet binder[5]	2.00	2.00	2.00
Choline chloride	0.03	0.03	0.03

[1] All ingredients (except soybeans) were donated by Delta Western Feed Mill (Indianola, MS) except carboxymethylcellulose (purchased from U.S. Biochemical, Cleveland, OH; lot no. 68258), catfish oil, donated by Protein Products (Sunflower, MS), and choline chloride (purchased from Fisher Scientific, Pittsburgh, PA; lot no. 8724).

[2] Mineral mix provided the following (g/100 g mix): manganese, 10; zinc, 20; iron, 7; copper, 0.7; iodine, 0.24; cobalt, 0.01; calcium (carrier).

[3] Provides 15% ascorbic acid activity as L-ascorbyl-2-polyphosphate (Roche Animal Nutrition, Nutley, NJ).

[4] Ascorbate-free vitamin mix provided the following (mg/kg diet): retinyl acetate, 1.51; cholecalciferol, 0.055; all-*rac*-α-tocopherol, 60; vitamin B-12, 0.01; riboflavin, 13.2; niacin, 88; *d*-pantothenic acid, 35.0; menadione dimethylpyrimidinol bisulfite, 4.4; folic acid, 2.2; pyridoxine hydrochloride, 11.0; thiamin, 11.0; selenium, 0.1.

[5] Carboxymethylcellulose.

search and Extension Center) were used for this study. Three hundred fish of mixed sex weighing approximately 3 g per fish were used. The experimental design employed in this study is traditionally used to determine feed efficiencies of experimental test diets in catfish. Fish were grown in 120-L glass aquaria with 20 fish/tank. There were five aquaria (tanks) or replicates per treatment. The flow-through system provided fresh well water at a rate of approximately 1 L/min, and water was monitored for potential contaminants. Water temperature, dissolved oxygen and pH were monitored at regular intervals during the study.

Fish were fed diets at an initial rate of approximately 4 g/100 g body wt. The feeding rate was adjusted weekly based on observations of consumption during the previous week through the end of the test. In this manner, rates that approximated satiation were maintained. Fish were weighed at initiation and at wk 2, 6 and 10. This was accomplished by transferring the fish from the test tank into a pre-weighed bucket containing enough water to hold the fish for a short time. On wk 2, 6, and 10, feed consumption was quantified by feeding a known quantity of feed to fish until they were satiated. Feed consumption was calculated by adjusting the starting amount for unfed pellets and for uneaten pellets removed from the tanks. The cumulative gain-feed ratio was estimated at wk 2, 6 and 10 by dividing the total gain by the sum of the feed offered to that point. The gain-feed ratio was adjusted for mortalities by adding to the total gain the estimated weight gain of fish that died during the experiment. In cases where fish died during the experiment and little or no remains were recovered, the weight of the fish was estimated based on the last known average fish weight. At the end of the study, three or more fish were randomly selected from each tank and filleted. The edible tissue (fillets) was composited and subjected to proximate analysis (moisture, protein, fat, ash) using AOAC methods (AOAC 1990).

Analysis of variance and Duncan's multiple range test (Duncan 1975) were used to compare results from all measured variables for each experimental unit (tank) (SAS 1989).

Dairy cattle study. Thirty-six multiparous Holstein cows (Monsanto Dardenne Technical Center, Dardenne, MO) between 93 and 196 d in lactation at the start of treatments were allotted to one of two blocks based on availability. Cows in block 1 started pretreatment 14 d before those in block 2. The pretreatment period lasted 14 d, during which all cows were fed a total mixed diet containing commercially available soybeans (A5403, Asgrow, Kalamazoo, MI) to allow for adaptation to a diet containing raw soybeans. Cows were then given the test diets for the remainder of the study. Within blocks, cows were assigned randomly to a total mixed diet containing one of the three test soybean lines.

Throughout the pretreatment and treatment periods, cows were given free access to a total mixed diet (**Table 3**). All diets were formulated to meet or exceed the nutrient recommendations of the National Research Council (1989) and provided similar quantities of crude protein, net energy, lipids and major minerals while maintaining equal proportions of soybeans on a dry matter basis. Soybeans were cracked daily (Davis Krimper-Kracker Roller Mill, Bonner Springs, KS) prior to blending with other dietary components.

Feed was offered twice daily so that fresh feed was available after each milking. Refusals were collected and weighed prior to the morning milking. Cows were housed in a tie-stall barn and released into an exercise lot prior to each milking in the parlor. Cows were observed at least twice daily, and any health-related observations were recorded.

During the last week of the pretreatment period and the first 3 wk of the treatment period, a composite milk sample was collected at four consecutive milkings beginning with the Sunday evening milking. Samples collected Sunday evening and Monday morning were preserved with potassium dichromate and analyzed for lac-

TABLE 3

Ingredient composition and proximate analysis of diets for dairy cow study

	Soybean line		
	A5403	GTS 61-67-1	GTS 40-3-2
	g/100 g dry matter		
Alfalfa hay	35.3	35.3	35.3
Corn silage	17.5	17.5	17.5
Raw soybeans			
Line A5403	10.2	0	0
Line GTS 61-67-1	0	10.2	0
Line GTS 40-3-2	0	0	10.2
Grain mix			
Ground corn	18.0	18.0	18.0
Soy hulls	0.8	0.8	0.8
Wheat midds	3.2	3.2	3.2
Dehulled soybean meal	12.4	12.4	12.4
$CaCO_3$	0.5	0.5	0.5
Ca_2PO_4	0.6	0.6	0.6
NaCl	0.4	0.4	0.4
Molasses	0.7	0.7	0.7
Vitamins and minerals[1]	0.4	0.4	0.4
Nutrient composition			
Dry matter[2]	69.63	68.84	68.77
Crude protein	21.62	21.05	21.50
Acid detergent fiber	21.27	21.68	22.92
Neutral detergent fiber	31.89	33.31	35.93
Ether extract	2.93	2.63	2.95
Ash[3]	4.15	3.95	4.12[1]

[1] Vitamin mix and minerals in the diet included calcium carbonate, dicalcium carbonate, magnesium oxide, magnesium potassium sulfate, selenium, sodium chloride, retinyl acetate, all-*rac*-α-tocopherol, cholecalciferol and trace minerals. Added vitamins and selenium resulted in 0.3 mg selenium/kg feed dry matter, 1.84 mg vitamin A/kg feed dry matter, and 0.047 mg cholecalciferol/kg feed dry matter.

[2] Expressed as the percentage of wet weight.

[3] Sum of concentrations of calcium, phosphorous, magnesium, potassium, sodium and sulfur.

tose (Mid-American Dairyman, St. Louis, MO). Samples collected Monday evening and Tuesday morning were preserved with bromopol and analyzed for fat and protein using a Foss-Milk-O-Scan, and somatic cell count was measured using a Foss-O-Matic machine (Foss Food Technology, Eden Prairie, MN). These analyses were performed at Dairy Lab Services (Dubuque, IA).

Cows were weighed after the morning milking on d−1, 0, 28 and 29. Also on these days, body condition was scored (Wildman et al. 1982), independently, by two scorers.

Total collection of urine and feces was conducted during the fourth week of treatment to determine dry matter digestibility and nitrogen balance. During the total collection period, cows remained in tie-stalls throughout the day and were milked with portable milkers. Urinary catheters (IMEX Foley #26FR/CH, TFX Medical, Alpharetta, GA) were inserted into the bladder 2 d before the start of the collection, and urine was collected into a carboy containing 500 mL/d of concentrated hydrochloric acid. Feces were collected into pans placed in the gutter behind each cow. Collection of urine and feces began after the morning milking on d 21. Each day, urine and feces were weighed and samples taken (2% feces and 0.25% urine). Also, 10% of each cow's ort was sampled daily. Samples of orts, urine and feces were stored frozen and composited at the completion of the 1-wk collection period. A representative milk sample was collected without preservative at each milking during the total collection and stored at −80°C. Milk and urine were analyzed for nitrogen using an automated analyzer (Leco FP-428 Nitrogen Determinator System, Leco Corp., St. Joseph, MI). Samples of feed offered to each group were collected daily throughout the total collection period; these samples, as well as feces and orts, were dried and ground to pass through a 2-mm screen for proximate analyses (Livestock Nutrition Lab Services, Columbia, MO).

On d 29, ruminal fluid samples were collected via stomach tube at approximately 1.5, 3 and 6 h after the morning feeding. Samples were strained through cheesecloth, and sulfuric acid was added to a concentration of 0.2 mol/L. Samples were stored frozen at −20°C and subsequently analyzed for volatile fatty acids by gas-liquid chromatography (30 m × 0.32 mm i.d. J&W capillary column coated with 0.25 μm of OV-351 phase; column temperature 140°C at 2°C/min; helium gas at 29 cm/s; detector gases hydrogen and air; optimized) using a gas chromatograph (model 5890, Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector. Acids were corrected for recovery of internal standard. Ruminal ammonia was measured by the phenol-hypochlorite colorimetric assay (Weatherburn 1967).

Data were analyzed by ANOVA using a randomized block design. A covariate was used in the model when appropriate. Due to animal availability, there was a confounding effect between blocks and day of lactation at the start of treatment. Day of lactation ranged from 122 to 196 d for cows in the first block and from 93 to 135 d for cows in the second block. Because of the confounding, deviations from block means during the 2-wk pretreatment period were used as the covariate. Differences were declared significant at $P < 0.05$. The model used included the covariate, design block, and treatment × block interaction. When the block × treatment interaction had $P < 0.25$, this term was dropped from the model. The model for all variables from the total collection period contained deviation from block mean for pretreatment milk production as a covariate.

One cow (fed A5403) was off-feed and had reduced milk production during the study. She was diagnosed with coliform mastitis and was removed from the study. There is no indication that her condition was treatment

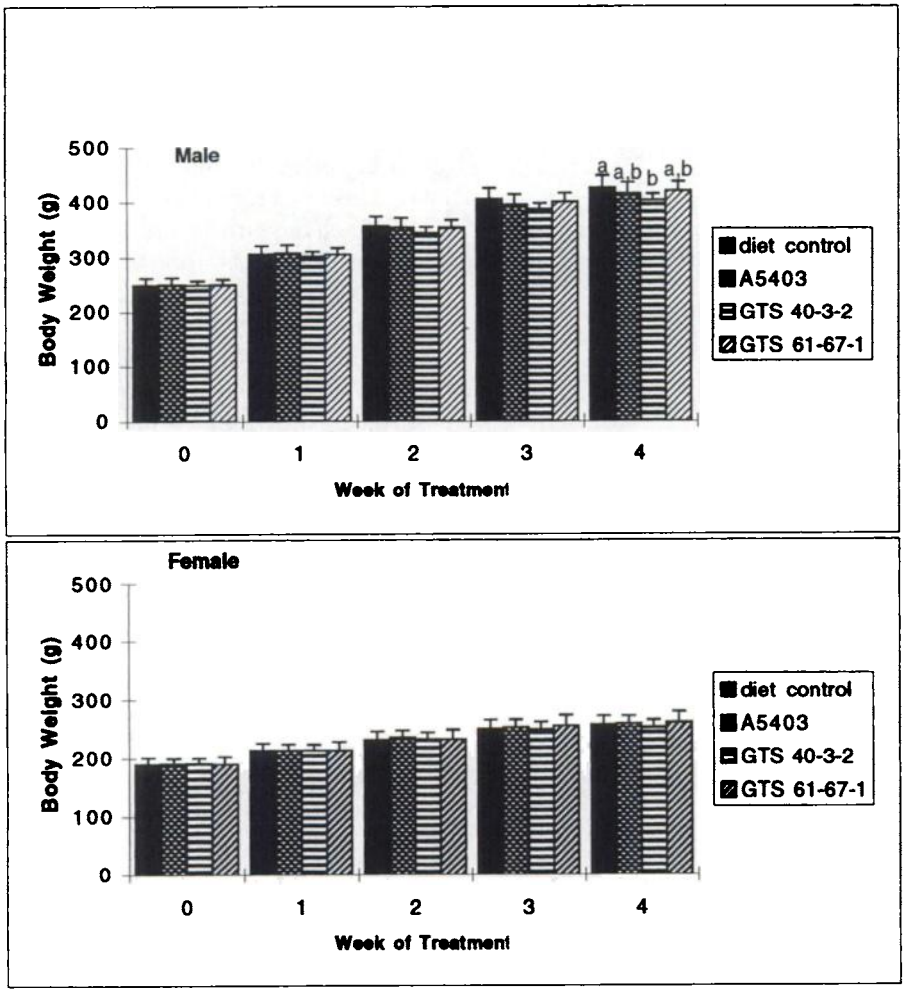

FIGURE 1 Mean weekly body weights of rats fed processed soybean. Values are means ± SD, $n = 10$. Rats were fed 24.8 g/100 g processed GTS (lines 40-3-2 or 61-67-1) or parental line (A5403) soybean meal in the diet. Diet control rats were fed Purina Laboratory Rodent Chow 5001 diet. Values with unlike superscripts are significantly different ($P > 0.05$). Shown are body weights of males (*upper panel*) and females (*lower panel*) fed diets containing processed soybeans.

related, and data from this cow were not included in the statistical analyses. Additionally, composite fecal samples were mislabeled for two cows. These samples were discarded, and data for these two cows are not included in analyses for the total collection period.

RESULTS

Rat studies. All test animals appeared healthy during the studies, and there were no meaningful differences in body weight, cumulative body weight gain, or food consumption (data not shown) between rats fed processed soybean meal (**Fig. 1**) or unprocessed ground meal (**Fig. 2**) relative to the parental line and GTS lines. When compared with results for the diet control group (fed commercial rat diet, not parental soybean line diet), body weights and cumulative body weight gains were slightly lower ($P < 0.05$) in male rats fed processed meal from GTS line 40-3-2 (Fig. 1) but not in females. Food consumption was slightly lower for males fed meal from both GTS line 40-3-2 ($P < 0.01$) and the parental line ($P < 0.05$) when compared with diet control males. These reductions are attributable to differences in processing conditions that may have affected the palatability of the diets: soybeans from the GTS and parental lines were processed at a pilot plant facility (Texas A&M University), unlike the diet control, which was processed on a commercial scale.

There were no differences in absolute or relative organ weights for treated and control animals of either sex fed processed meal (data not shown). Several differences ($P < 0.05$) in relative organ weights were observed for males fed GTS or parental line ground soybeans (unprocessed) compared with diet control males. These differences (13% greater relative kidney weight, 5 g/100 g GTS lines; 12% greater relative testes weight, 10 g/100 g parental line) were either not dose related (no differences at 10 g/100 g level) or were limited to the parental line only. Therefore, they were not considered to be related to genetic modification.

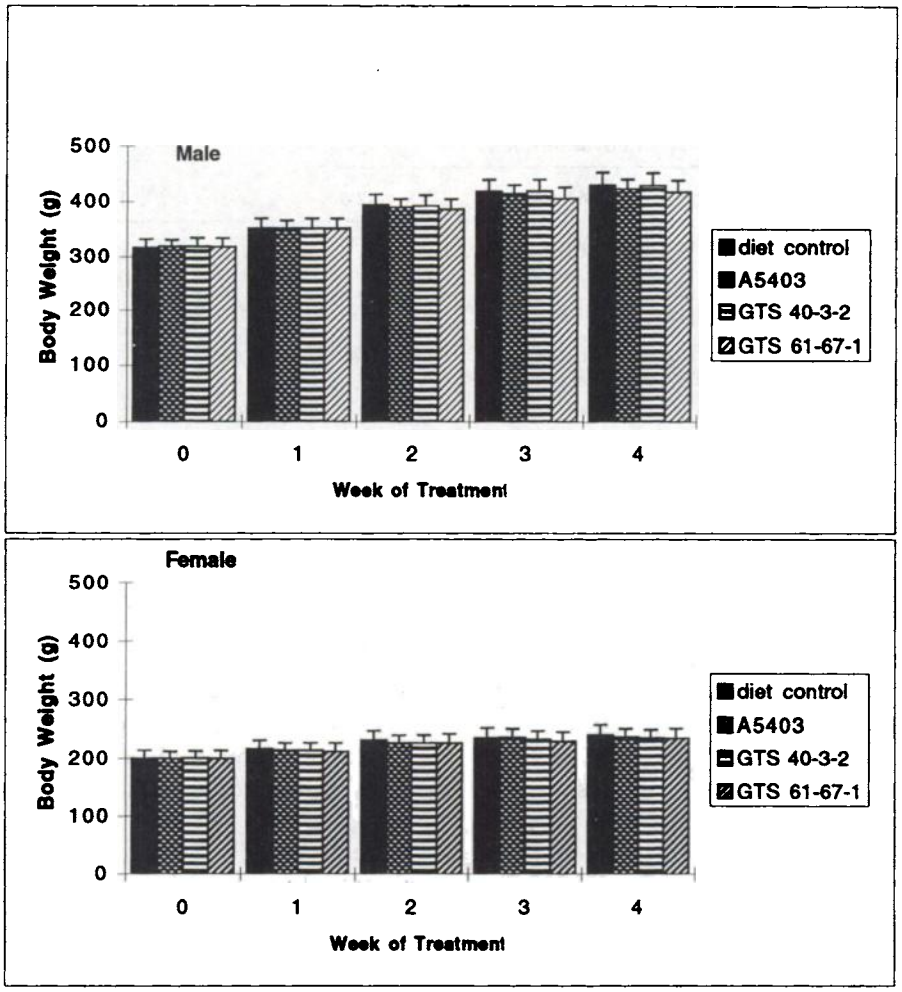

FIGURE 2 Mean weekly body weights of rats fed ground soybean. Values are means ± SD, $n = 10$. Rats were fed 10 g/100 g ground soybeans from GTS (lines 40-3-2 or 61-67-1) or the parental line (A5403). Diet control rats were fed Purina Laboratory Rodent Chow 5001 diet. There were no differences ($P > 0.05$). Shown are body weights of males (*upper panel*) and females (*lower panel*) fed diets containing ground soybeans.

There were no gross pathologic findings observed at necropsy that were considered related to genetic modification. However, the livers of several animals (males predominately) fed GTS and parental-line ground soybeans appeared a darker brown at necropsy; the liver of one diet control male also appeared darker. Because rats fed processed GTS and parental-line soybean meal did not exhibit a similar incidence of darker brown livers at necropsy, this finding may have been related to feeding rats high dietary levels of ground soybeans. Because this finding occurred both in rats fed ground GTS and in rats fed ground parental-line soybeans, it was not considered to be related to genetic modification. Except for the darker brown color, livers appeared normal at necropsy, and absolute and relative liver weights were comparable for GTS, parental-line soybean and diet control groups.

Minimal to mild common microscopic findings (e.g., inflammation and acinar cell apoptosis) were observed in the pancreas of animals from the unprocessed GTS, parental-line soybean and diet control groups. Because the incidence and severity of changes were comparable for all groups, these changes were not considered to have any relationship to diet. The absence of pancreatic hypertrophy in this study may be explained by the fact that protein from raw soybeans accounted for only approximately 17 g/100 g of the total protein in the diet. The ratio of milligrams of trypsin inhibitor to grams of protein in the diet was calculated to be similar to that of other diets containing soybean protein isolate that did not induce pancreatic hypertrophy when fed to rats for 4 wks (Gumbmann et al. 1986).

Poultry study. For the cumulative study period (d 0–42), there were no differences ($P < 0.05$) among groups for body weight, live weight gain, feed intake, gain:feed ratio or livability (**Table 4**). As expected, males were heavier, consumed more feed, and had better gain:feed ratios but lower livability than females, regardless of the source of soybean meal tested.

There were no differences among groups in breast muscle and fat pad weights either as absolute weights or as proportions of body weight (Table 4), and there

TABLE 4

Body weight gain, feed consumption, gain:feed ratio, livability, breast and fat pad weight during the 42-d study period in chickens fed glyphosate-tolerant soybeans (GTS) or parental line processed soybean meal[1]

	Soybean line				Sex		
	A5403	GTS 61-67-1	GTS 40-3-2	SEM	Female	Male	SEM
Number	120	120	120	N/A	180	180	N/A
Body weight, *g*	2192	2188	2144	18	2041[a]	2309[b]	15
Daily gain, *g/d*	51	51	50	0.4	48[a]	54[b]	0.4
Daily feed consumed, *g/d*	93	93	92	0.8	88[a]	97[b]	0.6
Gain:feed, *g/g*	0.551	0.548	0.546	0.003	0.541[a]	0.556[b]	0.002
Livability, %	90.8	89.2	91.7	2.8	93.9[d]	87.2[c]	2.3
Breast weight, *g*	302	296	294	0.004	284[a]	311[b]	0.003
Breast/body weight, *g/100 g*	13.8	13.5	13.7	0.14	13.9[b]	13.4[a]	0.11
Fat pad weight, *g*	81	82	77	0.003	85[b]	75[a]	0.002
Fat pad/body weight, *g/100 g*	3.7	3.8	3.6	0.11	4.2[a]	3.3[b]	0.09

[1] Values are means, n = 60 birds/(sex·group). [a,b] Values with unlike superscripts are significantly different ($P \leq 0.01$). [c,d] Values with unlike superscripts are statistically different ($P \leq 0.05$). N/A = not applicable.

were no soybean × sex interactions ($P \geq 0.05$) for the variables measured. Males had heavier breast muscle weights and lighter fat pad weights than females. Breast muscle and fat pad weights as proportions of body weight were higher for females than for males.

Thirty-four birds were culled from the study or died. Known reasons for removal were ascites, sudden death syndrome and twisted leg. These anomalies are generally recognized to be associated with the rapid weight gains of commercial broilers (Julian 1992, Randall 1991) and were not related to treatment.

Catfish study. The overall health and survival of fish in the study were very good and were comparable among groups (**Table 5**). Fish in one of the tanks fed line 61-67-1 became infected with Edwardsiella ictaluri. This disease, enteric septicemia, is common in catfish, and the outbreak was not related to genetic modification. To prevent the spread of this virulent pathogen to other unaffected tanks, the fish in this tank were removed and the tank sterilized.

Gain:feed ratios were not different among fish fed the parental line A5403 diet and those fed GTS line 61-67-1 or 40-3-2 (Table 5). The proportionate weight gains of fish fed the GTS lines were not different from that of fish fed the parental-line diet. The proportionate weight gain of fish fed GTS line 61-67-1 was higher ($P < 0.05$) than that of fish fed GTS line 40-3-2. This difference was due to the fact that fish fed line 61-67-1 consumed more feed and converted the feed slightly better than fish fed line 40-3-2.

Body composition data from the three groups were not different when expressed on a wet-weight basis (**Table 6**). There were no differences in moisture, protein, fat or ash among fish regardless of dietary treatment.

Dairy cow study. Animal health throughout the study generally was good. Loose stools were observed in several cows regardless of the soybean source and likely were related to the large amount of raw soybeans fed in this study.

Least-square means for milk production (kg/d) were not affected by soybean source (**Table 7**). However, 3.5% fat-corrected milk production was 2.5 and 2.7 kg/d higher for cows fed GTS lines 61-67-1 and 40-3-2, respectively ($P < 0.05$), compared with cows fed the parental line ($P < 0.02$). Fat-corrected milk is calculated from milk production and milk fat percentage, neither of which differed in this study. Likewise, fat-corrected milk per unit of energy intake was not affected by diet. Milk composition was not affected by the three soybean sources fed in this study (Table 7).

Dry matter and net energy intakes (**Table 8**) were not affected by the source of soybeans used in the total

TABLE 5

Feed consumption, feed conversion, weight gain, final body weight and survival of catfish fed diets with and without glyphosate-tolerant soybean (GTS) lines[1]

	Soybean line		
	A5403	GTS 40-3-2	GTS 61-67-1[2]
Feed consumption, *g/fish*	22.1 ± 0.30[b]	21.8 ± 0.42[b]	23.7 ± 0.40[a]
Gain:feed, *g/g*	0.89 ± 0.01	0.85 ± 0.01	0.89 ± 0.02
Weight gain,[3] *g/100 g initial wt*	673 ± 16.5[ab]	630 ± 11.7[b]	700 ± 25.8[a]
Final weight, *g*	22.6 ± 0.4[b]	21.8 ± 0.4[b]	24.5 ± 0.8[a]
Survival, %	99 ± 1.0	97 ± 1.2	95 ± 1.2

[1] Values are means ± SEM for five replicate tanks containing 20 fish/tank. Within a row, values with different letters are significantly different ($P \leq 0.05$).

[2] For GTS 61-67-1, there were four replicate tanks due to the loss of one tank from infection.

[3] Initial fish weights were 2.93 g (A5403), 2.98 g (GTS 40-3-2) and 3.06 g (GTS 61-67-1).

TABLE 6

Proportions of moisture, protein, fat and ash of fillets of catfish fed diets with and without glyphosate-tolerant soybean (GTS) processed meal[1]

	Soybean line		
	A5403	GTS 61-67-1	GTS 40-3-2
	g/100 g wet wt		
Moisture	79.6 ± 0.2	79.9 ± 0.2	79.7 ± 0.2
Protein	16.8 ± 0.1	16.9 ± 0.1	16.9 ± 0.1
Fat	1.6 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
Ash	1.2 ± 0.01	1.2 ± 0.02	1.2 ± 0.02

[1] Values are means ± SEM. A minimum of three fish per tank were filleted and composited, and proximate composition was determined using AOAC methods. The means were calculated from composite determinations for each replicate tank. No significant differences were observed ($P \geq 0.05$).

mixed diets, and apparent dry matter digestibility was similar for the three diets. Similarly, indices of nitrogen balance were not affected by diet. On average, cows consumed 2.4 kg/d of raw soybeans. Most nutritionists recommend that cows be fed no more than 2.3 kg soybeans/d due to the soybean lipid content (Emery and Herdt 1991).

Molar proportions of volatile fatty acids in the rumen (**Table 9**) were not affected by source of soybeans. Similarly, ruminal ammonia was unaffected. These analytes are critical products of rumen fermentation and

TABLE 7

Milk production, milk composition, dry matter and net energy intakes during the entire treatment period of cows fed parental line or glyphosate-tolerant soybeans (GTS)[1]

	Soybean line		
	A5403	GTS 61-67-1	GTS 40-3-2
Number	11	12	12
Milk, *kg/d*	34.9 ± 0.5	36.2 ± 0.5	36.2 ± 0.5
3.5% fat-corrected milk, *kg/d*	34.1 ± 0.8[a]	36.6 ± 0.7[b]	36.8 ± 0.7[b]
Fat, *g/100 g*	3.37 ± 0.09	3.62 ± 0.08	3.59 ± 0.09
Protein, *g/100 g*	3.28 ± 0.03	3.29 ± 0.03	3.23 ± 0.03
Lactose, *g/100 g*	4.72 ± 0.03	4.75 ± 0.03	4.72 ± 0.03
Somatic cell count,[2] $\times 10^{-3}$	110 ± <1	59 ± <1	93 ± <1
Dry matter intake, *kg/d*	24.4 ± 0.3	25.4 ± 0.3	24.7 ± 0.3
Net energy intake,[3] *MJ* NE_L*/d*	167.8 ± 4.6	180.4 ± 4.2	179.6 ± 4.2
FCM/NE_L intake,[3] *kg/MJ*	0.19 ± 0.01	0.21 ± 0.01	0.21 ± 0.01

[1] Values are least square means ± SEM. Values with unlike superscripts are significantly different ($P < 0.05$).

[2] Values are antilog of least-squares mean from analysis of logs of somatic cell counts.

[3] FCM = fat-corrected milk, NE_L = net energy lactation.

TABLE 8

Milk production, feed intake, dry matter digestibility and nitrogen balance during the 7-d total collection period of cows fed commercial or glyphosate-tolerant soybeans (GTS)[1]

	Soybean line		
	A5403	GTS 61-67-1	GTS 40-3-2
Number	10	12	11
Milk, *kg/d*	33.6 ± 0.9	35.5 ± 0.8	34.4 ± 0.9
Dry matter intake, *kg/d*	23.8 ± 0.7	25.7 ± 0.7	23.8 ± 0.7
Nitrogen intake, *g/d*	840 ± 16	874 ± 15	851 ± 16
Dry matter digestibility, %	69.0 ± 0.7	69.4 ± 0.6	68.6 ± 0.7
Milk nitrogen, *g/d*	214 ± 7	230 ± 6	214 ± 6
Urine nitrogen, *g/d*	446 ± 10	466 ± 9	467 ± 9
Fecal nitrogen, *g/d*	236 ± 9	248 ± 8	240 ± 9
Absorbed nitrogen, *g/d*	597 ± 22	641 ± 19	601 ± 20
Retained nitrogen, *g/d*	−68 ± 19	−55 ± 17	−76 ± 18
Productive nitrogen, *g/d*	147 ± 21	175 ± 19	138 ± 20

[1] Values are least square means ± SEM. Treatment means were not significantly different ($P \geq 0.05$).

are affected by nutrient composition of the feed consumed and additives that may inhibit or promote the growth of certain microorganisms.

DISCUSSION

The growth and gain-to-feed performance of animals fed GTS meal sources was comparable to those of ani-

TABLE 9

Ruminal volatile fatty acids and ammonia nitrogen of cows fed commercial or glyphosate-tolerant soybeans (GTS)[1]

	Soybean line		
	A5403	GTS 61-67-1	GTS 40-3-2
Number	11	12	12
Acetate, *mol/100 mol*	70.75 ± 1.09	69.95 ± 1.05	70.88 ± 1.05
Propionate, *mol/100 mol*	20.71 ± 1.05	21.74 ± 1.01	20.62 ± 1.01
Isobutyrate, *mol/100 mol*	0.07 ± 0.04	0.03 ± 0.03	0.11 ± 0.03
Butyrate, *mol/100 mol*	7.89 ± 0.21	7.74 ± 0.20	7.55 ± 0.20
Isovalerate, *mol/100 mol*	0.20 ± 0.07	0.09 ± 0.07	0.31 ± 0.07
Valerate, *mol/100 mol*	0.43 ± 0.12	0.44 ± 0.12	0.53 ± 0.12
Acetate:propionate, *mol/mol*	3.50 ± 0.22	3.35 ± 0.21	3.59 ± 0.21
Ammonia N, *mol/L*	0.111 ± 0.006	0.112 ± 0.006	0.116 ± 0.006

[1] Values are least square means ± SEM. Treatment means were not significantly different ($P > 0.05$). All values are from analysis conducted using means of samples collected at 1.5, 3 and 6 h after feeding.

mals fed parental-line soybeans. Milk production and composition, rumen fermentation and nitrogen digestability were likewise comparable in dairy cows fed both GTS lines and the parental line. Breast muscle and fat pad weights were similar for all groups of chickens, as were proximate analyses of catfish fillets from all groups fed GTS and parental soybean lines. The lack of material differences in the various measured variables for the diverse animal species fed GTS and parental-line soybeans confirms that the GTS are equivalent to the parental soybeans for use in animal feeds. The catfish and broiler studies were considered to be the most sensitive tests to detect small differences in the wholesomeness or nutritional value of the soybeans. The replication employed in the broiler study made it possible to detect a 3.5% difference in gain and a 2% difference in the gain:feed ratio among the groups. The actual numerical differences observed among groups in the study were lower than these. The increases in growth for catfish and broilers (approximately 700% and 5000%, respectively) experienced during the study duration (e.g., a 45-g chick at study initiation weighed more than 2200 g at the end of the 6-wk growth study) meant that these animals would be sensitive to changes in the nutrient value of the diets. The protein requirement of broilers is significantly higher than those of other food animals such as swine (NRC 1988 and 1994).

Normally, animal feeding studies are not used to evaluate the quality of new varieties of soybeans that are generated via conventional plant breeding. Compositional analysis of new soybean varieties is considered sufficiently sensitive to assess nutritional quality, and this practice has worked well through the years. Although the animal feeding studies provide some reassurance that no major changes occurred in the genetically modified soybeans, in reality, this was already established by the compositional analyses. It has been recommended that primary reliance be given to thorough compositional analyses to detect potential material differences for new genetically modified lines that may be developed (International Food Biotechnology Council 1990, U.S. Food and Drug Administration 1992).

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