Food and Chemical Toxicology 46 (2008) 3808–3817

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Subchronic feeding study of high oleic acid soybeans (Event DP-3Ø5423-1) in Sprague–Dawley rats

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ARTICLE INFO

Article history:
Received 16 May 2008
Accepted 1 October 2008

Keywords:
Biotechnology
Genetically-modified
High oleic acid oil
Rat
Soybean
Subchronic

ABSTRACT

DP-3Ø5423-1 (305423) is a genetically-modified (GM) soybean that was produced by biolistic insertion of a *gm-fad2-1* gene fragment and the *gm-hra* gene into the germline of soybean seeds. The *gm-fad2-1* gene fragment cosuppresses expression of the endogenous *FAD2-1* gene encoding the seed-specific omega-6 fatty acid desaturase resulting in higher concentrations of oleic acid (18:1) relative to linoleic acid (18:2). The *gm-hra* gene encoding a modified acetolactate synthase (ALS) enzyme was used as a selectable marker. In the current study, processed fractions (meal, hulls, and oil) from 305423 soybeans, non-GM soybeans with a similar genetic background (near isoline control) and three commercially-available non-GM varieties were used to formulate diets that were nutritionally comparable to PMI® Certified Rodent LabDiet® 5002. Diets were fed to young adult Crl:CD(SD) rats (12/sex/group) for approximately 90 days. Compared with rats fed the non-GM control diet, no biologically relevant differences were observed in rats fed the 305423 diet with respect to body weight/gain, food consumption/efficiency, mortality, clinical signs of toxicity, or ophthalmological observations. No test diet-related effects were observed on neurobehavioral assessments, organ weights, or clinical or anatomic pathology. These results demonstrated that 305423 soybeans are as safe and wholesome as non-GM soybeans.

1. Introduction

Assessment of the safety of foods obtained from genetically-modified (GM) crops is based on the concept of substantial equivalence that was originally proposed by OECD (1993). This paradigm was developed to account for the lack of data to demonstrate that whole foods can be proven to be safe in an absolute sense despite the fact that they have been used safely for as long as foods themselves have been consumed. Since the concept of substantial equivalence was originally proposed, numerous international scientific authorities have published guidelines by which to assess substantial equivalence between GM crops and their non-GM comparators (Codex, 2003b, 2007; EC, 1997, 2003a,b, 2004; EFSA, 2006a,b; FAO, 1996, 2000; ILSI, 2003b, 2004; Jonas et al., 1996; OECD, 1997, 2003; WHO, 1991, 1995).

The principles of substantial equivalence assessment have developed into a systematic approach that focuses on comparing a particular GM crop to the nearest isogenic relative using agronomic metrics and compositional analysis to determine if genetic modification produced unintended pleiotropic effects (Sidhu et al., 2000; OECD, 2001, 2002; Ridley et al., 2002; ILSI, 2004; Obert et al., 2004; Herman et al., 2007). However, it is possible that the process of genetic modification could result in unintended, potentially adverse pleiotropic changes that might not have been detected analytically (Delaney, 2007). Therefore, the nutritional quality of food and feed fractions from GM crops has been assessed using feeding studies with broiler chickens and rats (Taylor et al., 2003a,b,c, 2005; Hammond et al., 2006a,b; Hammond et al., 2004; MacKenzie et al., 2007; McNaughton et al., 2007; Malley et al., 2007; Appenzeller et al., 2008).

This paper reports the outcome of a repeated dose feeding study was conducted in rats to compare the nutritional quality of event DP-3Ø5423-1 (305423) soybeans with that of non-GM soybeans following subchronic dietary exposure using growth performance and toxicology response variables (OECD 1998). Event DP-3Ø5423-1 (305423) is a GM soybean (*Glycine max*) that was produced by biolistic co-transformation of a *gm-fad2-1* gene fragment and the *gm-hra* gene, both originating from soybean. Transcription of the *gm-fad2-1* gene fragment cosuppresses expression of the endogenous soybean *FAD2-1* gene which encodes a seed-specific omega-6 fatty acid desaturase enzyme that catalyzes desaturation of oleic acid (18:1) to linoleic acid (18:2; Okuley et al., 1994; Heppard et al., 1996; for a comprehensive review of cosuppression and the applications of

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doi:10.1016/j.fct.2008.10.003

RNAi in agricultural biotechnology see Small, 2007). Correspondingly, suppression of *FAD2-1* activity results in increased proportions of oleic acid and decreased levels of linoleic acid, linolenic acid, and to a lesser extent, palmitic acid in the seed storage lipid relative to non-GM soybeans (Buhr et al., 2002; Stoutjesdijk et al., 2002). The high oleic fatty acid profile confers higher oxidative stability, reduced concentrations of saturated fat, and eliminates the need for trans-hydrogenation. A highly resistant allele of the acetolactate synthase gene of soybeans (*gm-hra*) was produced by isolating the herbicide-sensitive acetohydroxyacid synthase (*ahas*) gene from soybean and changing the coding sequence for two specific amino acids (Mazur and Falco, 1989; Green, 2007). AHAS enzymes catalyze the first common step in the biosynthesis of the branched chain amino acids (Ile, Leu, Val; LaRossa and Schloss, 1984; LaRossa and Falco, 1984; Duggleby and Pang, 2000). This enzyme is the primary site of action of a number of classes of herbicides including those in the sulfonylurea and imidazolinone classes (LaRossa and Schloss, 1984; Chaleff and Mauvais, 1984; Tan et al., 2006). Expression of the GM-HRA protein was used in 305423 soybeans as a selectable marker during the transformation process because it confers tolerance to the sulfonylurea and imidazolinone classes of herbicides. The oil produced from this newly-developed soybean will be marketed as TREUS™ High Oleic Soybean Oil.

2. Materials and methods

2.1. Test, control, and reference substances

Event DP-3Ø5423-1 soybean (305423), its non-transgenic near isoline control (091; same genetic background as 305423, but does not contain the *gm-fad2-1* gene fragment or the *gm-hra* gene, and three non-transgenic commercially-available reference soybean varieties (93B86, 93B15, and 93M40) were produced in isolated field plots at the Pioneer research center near Santiago, Chile during the 2005/2006 growing season. Bulked and ground raw soybean samples from each of the sources were subjected to qualitative real-time polymerase chain reaction (qRT-PCR) analysis utilizing a primer set specific for DP-3Ø5423-1 to confirm the molecular identity of the test variety and to demonstrate that that transgenic event was not present in the control (091) or reference soybeans (E.I. DuPont de Nemours and Company, Wilmington, DE). Additionally, an enzyme-linked immunosorbent assay (ELISA) was performed to confirm expression of the GM-HRA protein in ground 305423 soybean samples and verify that this protein was not present in the control or reference soybean samples (Pioneer, a DuPont Company, Johnston, IA).

2.2. Soybean processing and composition analysis

Control, reference, and test soybeans were batch-processed sequentially into feed fractions (dehulled/defatted toasted meal, toasted ground hulls, and degummed, alkaline-refined oil) at GLP Technologies (Navasota, TX). Meal samples were analyzed by Pope Testing Laboratories Inc. (Irving, TX) for quality indicators (crude protein, protein dispersibility index, protein solubility, crude fat, and urease activity) to verify suitability for use in diets. Meals and ground hulls were further analyzed for nutrient composition (proximates, fiber [crude, neutral detergent, acid detergent], fat, amino acids, minerals [Ca, P, Mg, K, Na, Zn, Mn, Cu, Fe], vitamins [B1 (thiamine), B2 (riboflavin), folic acid]), and concentrations of anti-nutrients (trypsin inhibitor activity, lectin activity, phytic acid) and isoflavones (daidzein, genistein, and glycitein as free aglucone and glycoside conjugates) at EPL Bio-Analytical Services (EPL-BAS; Harristown, IL). Soybean oil samples were evaluated for relative percentage fatty acid composition including palmitic acid (16:0), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidonic acid (20:0), gadoleic acid (20:1), and behenic acid (22:0) at EPL-BAS. Additional vitamins (B6 [pyridoxine], niacin, pantothenic acid) and selenium were quantified at Woodson-Tenent Laboratories (Woodson-Tenent; Memphis, TN). Concentrations of aflatoxins B1, B2, G1, and G2, zearalenone, deoxynivalenol and its 3-acetyl and 15-acetyl derivatives, fumonisins B1, B2, and B3, and T-2 toxin were determined at Romer Laboratories Inc. (Romer; Union, MO).

2.3. Experimental diet formulation, composition, and molecular characterization

Fractions from test, control and reference soybeans were incorporated by Purina TestDiet (Richmond, IN) into rodent diets nutritionally comparable to PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002. Individual diets contained meal (20% w/w), hulls (approximately 1.5% w/w), and oil (1.5–1.7% w/w) and met LabDiet® 5002 specifications for protein and calorie content. Diets were refrigerated upon receipt by the testing facility (DuPont Haskell) until they were apportioned into food jars (see below). All diets were analyzed quantitatively for nutrient composition and contaminants (summarized in Table 1). Nutritional proximates, fiber, amino acids, fatty acids, minerals, and vitamins (including E [as α-tocopherol], β-, γ-, δ-, and total tocopherols) were measured at EPL-BAS. Gross energy values of the diets were determined by calculation (21 CFR 101.9). Additional vitamins (B12 [cobalamin], A [as retinol], D3 [cholecalciferol], β-carotene, choline, biotin), minerals (Se, Co, Cl, I, Cr, F), and heavy metals (As, Cd, Pb, Hg) were quantified at Woodson-Tenent (Memphis, TN). Mycotoxins (aflatoxins B1, B2, G1, and G2, zearalenone, deoxynivalenol and its 3-acetyl and 15-acetyl derivatives, fumonisins B1, B2, and B3, T-2 toxin, and moniliformin) were determined at Romer Labs (Union, MO); oosporein and ergot metabolites (ergosine, ergotamine, ergocornine, ergocryptine, and ergocristine) were determined at the University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO). Concentrations of pesticide and herbicide residues (chlorinated hydrocarbons [aldrin, BHC-α, -β, and -δ, chlordane, DDT-related substances, dieldrin, endrin, HCB, heptachlor and its epoxide, lindane, methoxychlor, mirex, and PCB], organophosphates [diazinon, disulfoton, ethion, malathion, methyl and ethyl parathion, thimet, thiodan {endosulfan}, and trithion], and the herbicide paraquat [gramoxone]) were determined at Columbia Food Laboratories (Corbett, OR). Samples from all diets were subjected to event specific qRT-PCR analysis for DP-3Ø5423-1 to confirm identity of rodent diets containing processed fractions from 305423 soybeans and to verify absence of detectable transgenic contamination of the control and reference diets.

2.4. Animal care and management

The feeding trial was conducted at DuPont Haskell Global Centers for Health and Environmental Sciences (Newark, DE). Four-week-old male and female Crl:CD(SD) rats were obtained from Charles River Laboratories Inc. (Raleigh, NC). Rats were housed individually in stainless steel, wire-mesh cages suspended above cage boards. Animal rooms were maintained at 22 °C ± 4 °C and relative humidity 50% ± 20%. An approximate 12-h light/dark cycle was provided by automated fluorescent illumination. Rats were provided tap water (United Water Delaware; Wilmington, DE) *ad libitum*. During quarantine and acclimation, rats were fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum* (Purina Mills, Richmond, IN). This study complied with US EPA FIFRA (40 CFR Part, 160) Good Laboratory Practice (GLP) Standards and the following guidelines designed for rodent subchronic toxicology studies: OECD, Section 4 (Part 408), Repeated-dose 90-Day Oral Toxicity Study in Rodents, *Guideline for the Testing of Chemicals* (OECD, 1998); US EPA, OPPTS 870.3100, 90-Day Oral Toxicity in Rodents, *Health Effects Test Guidelines* (US EPA, 1998); and Commission Directive 2001/59/EC, Part B.26, *Methods for the Determination of Toxicity* (EC, 2001).

2.5. Study design and diet administration

At study initiation, rats of each gender (7–8 weeks of age) were randomized by body weight into five experimental groups (12/sex/group) so that there were no statistically significant differences among group body weight means within a gen-

Table 1
Compositional analyses (all soybean sources).

Analyte	Seed	Meal	Hulls	Oil	Diet
Proximates (crude protein, crude fat, carbohydrate, ash)		X	X		X
Moisture and dry matter		X	X		X
Fiber (crude, ADF, NDF)		X	X		X
Fatty acids				X	X
Amino acids (essential and non-essential)		X			X
Minerals (Ca, P, Mg, K, Fe, Zn, Na, Cu, Mn, Se)		X			X
Minerals (Co, Cl, I, Cr, F)					X
Heavy metals (As, Cd, Pb, Hg)					X
Vitamin E (α-tocopherol), and β-, γ-, δ-, and total tocopherols				X	X
Vitamins (B1, B2, B6, folic acid, niacin, pantothenic acid)		X			X
Vitamins (β-carotene, B12, A, D3, biotin, choline)					X
Trypsin inhibitor		X	X		
Phytic acid		X			
Lectin activity		X			
Isoflavones (free and conjugated)		X			
Mycotoxin panel			X		X
Pesticide residue panel					X
Molecular event detection by PCR (DP-3Ø5423-1)	X	X	X		X
Protein detection by ELISA (GM-HRA)	X	X	X		

An "X" indicates that the individual analyte or classes of analytes were assessed in that particular fraction.