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Rat and poultry feeding studies with soybean meal produced from imidazolinone-tolerant (CV127) soybeans

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ABSTRACT

The safety and nutritional properties of CV127 soybeans were evaluated in rat and broiler feeding studies. Some episodic differences were observed between rats fed CV127, Conquista, and the standard diet for the endpoints examined. None of these differences were considered treatment related, adverse, or biologically meaningful. In general, birds fed diets containing CV127, Conquista, or Monsoy 8001 showed no significant differences in growth and performance response variables. Chickens fed diets containing Coodetec 217 had lower body weight and weight gain for all developmental periods compared to CV127, but no significant differences were found in feed conversion for the two diets during any development period. The results of both feeding studies demonstrate that CV127 soybeans are as safe, wholesome, and nutritionally valuable as the other soybean meals tested, including those varieties for which histories of safe use have been established and well documented.

1. Introduction

Genetically modified (GM) herbicide-tolerant crops have become an important tool in crop production practices. In 2011, approximately 80% of the total hectares of GM crops planted had herbicide resistance traits (Green, 2012) Imidazolinone herbicides control weeds by inhibiting the enzyme acetohydroxyacid synthase (AHAS), also called acetolactate synthase, which is a critical enzyme in plants for the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine.

Soybeans are an important global crop, and one of the most commonly modified by genetic engineering, used for both human food and animal feed. In 2010/2011, the main soybean producing countries were the United States (U.S.), Argentina, Brazil, and China (U.S. Soybean Export Council, 2015) Soybeans produced in the U.S. are almost exclusively GM crops (GM Compass, 2015) In the U.S., the majority of soybeans are processed using a hexane-extraction method, which removes the oil and results in the production of oil and meal. A crushed soybean produces about 79% meal, 18.5% oil, and 2.5% waste and hulls (U.S. Soybean Export Council, 2015) The

Abbreviations: AHAS, Acetohydroxyacid synthase; ALT, alanine aminotransferase; ALB, albumin; ALP, alkaline phosphatase; AST, aspartate aminotransferase; PLT, blood platelet count; BUN, blood urea nitrogen; Ca, calcium; CHOL, cholesterol; CREA, creatinine; GM, genetically modified; GMO, genetically modified organism; GLUC, glucagon; GLP, Good Laboratory Practices; HCT, hematocrit; HGB, hemoglobin; ITAL, Institute of Food Technology; ILSI, International Life Sciences Institute; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; AMEn, nitrogen-corrected apparent metabolizable energy; P, phosphorous; RBC, red blood cell; SP, Santo Antônio da Posse; TPROT, total protein; TRIG, triglycerides; U.S., United States; WBC, white blood cell.

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soybean oil is used in human foods, biodiesel production, and industrial applications; whereas, the soybean meal is primarily used as a source of protein in farm animal feed.

The soybean plant (*Glycine max* L.) under investigation in this publication is a variety derived from a single transformation event designated as BPS-CV127-9. This transformed soybean variety is tolerant to imidazolinone herbicides due to the introduction of an imidazolinone-tolerant AHAS large subunit gene (*csr1-2*) from *Arabidopsis thaliana* into the soybean plant genome. The *Arabidopsis* AHAS protein is a member of the class of AHAS (large subunit) proteins found ubiquitously in plants. The mutation in the *Arabidopsis ahas* gene responsible for imidazolinone herbicide tolerance is a single nucleotide change of guanine to adenine, which results in a codon change from AGT to AAT and a single amino acid substitution of serine to asparagine at position 653 of the AHAS protein. In addition, a second mutation, R272K, was identified when this gene was introduced into soybean without impacting the enzymatic function of the AHAS enzyme and which retains its normal biosynthetic function in the transgenic plant (unpublished data).

The introduction of genes into crops for agronomic optimization or other favorably desired features requires that the GM crop be as safe and as nutritious as the native or conventional varieties. This core requirement has therefore introduced the concept of substantial equivalence to the safety assessment of GM crops and their derived foods and feeds. As defined by various scientific and regulatory bodies, substantial equivalence comparisons include molecular, compositional, phenotypic, and agronomic analysis to identify commonalities and divergences between the GM crop and its isogenic or near isogenic counterpart, and hence ensure the absence of unintended differences with the potential to affect human and animal health, or the environment (OECD, 2003; EFSA, 2006) This comparative safety assessment approach may be supplemented with feeding studies, which are intended to further confirm the safety and nutritional wholesomeness of the GM crop, thereby providing added safety assurance.

Although compositional studies confirmed the equivalence of CV127 to commercial soybean varieties, animal feeding studies were undertaken to provide further support for commercial acceptance of this new soybean variety. A previous subchronic safety study with CV127 soybean meal reported no adverse effects when administered to Wistar rats at dietary incorporation levels of 11% or 33% (Chukwudebe et al., 2012) In the current paper, we report additionally on the safety and nutritional wholesomeness of CV127 soybeans in Sprague–Dawley rats following a 90-day exposure at dietary incorporation levels ranging from 7.5 to 30%. Because soybeans are a major source of protein in the diets of most farm animals, the nutritional wholesomeness of CV127 was also investigated by feeding the processed meal to broiler chickens over a 42-day period.

2. Materials and methods

2.1. 90-Day rat feeding study

2.1.1. Soybeans

The soybeans used to produce the soybean meals tested in this study were from the 2010/2011 crop season in Senador Canedo, Goiania State, Brazil. Soybeans were cultivated using standard agricultural practices.

Soybean samples were characterized by the laboratory of GeneScan-Eurofins in Indaiatuba, SP, Brazil with the use of event-specific PCR to detect the *csr1-2* (*ahasl)* gene that encodes the imidazolinone-tolerant enzyme AHAS. Following standard agronomic practices, the safety assessment of CV127 was based on biomarker comparisons to Conquista, the nearest isogenic relative based on derivation, agronomic performance metrics, and compositional analysis. Hence, the CV127 soybeans were compared directly to Conquista soybeans, the near isogenic control variety that was transformed to give CV127. Only CV127 showed a positive PCR, thereby verifying the identity of this lot of soybean, as well as the lack of presence of CV127 in Conquista soybeans.

Soybeans were reevaluated upon receipt at the testing facility for the presence of the soybean reference gene *lectin* and an event-specific gene by PCR. The *lectin* gene was detected in all samples, while the event-specific gene was detected only in CV127 soybeans, further confirming the identity of the soybeans. The soybeans were milled using a SFSP56x40 beater pulverizer (Muyang Group Company, Yangzhou, Jiangsu Province, China).

2.1.2. Animals

The rat feeding study was conducted at the Supervision and Testing Center for Genetically Modified Organisms (GMO) Food Safety, Ministry of Agriculture located in Beijing, China in compliance with GMO Plant and Its Food Safety Testing – 90 Days Rat Feeding Test (Chinese Standard NY/T 1102–2006, 2006) The Chinese standards referenced in this document are methodologically equivalent to analogous OECD, EPA and other international approaches. Male and female Sprague–Dawley rats, approximately 4–5 weeks old and weighing 80–100 g, were obtained from Vital River Laboratories (Beijing, China). Animals were acclimated to the laboratory environment for five days prior to study initiation. The animal rooms were maintained at a relative humidity of 40–60%, room temperature of 22–25 °C, and 12 h light/12 h dark cycle. Animals were provided *ad libitum* access to feed, standard rat maintenance diet (Ke-Ao-Xie-Li Feed Company, Beijing, China), and water in accordance with Chinese Standard (Chinese Standard GB14924.3–2001, 2001) Rats were group-housed for the entire study period (five animals per cage).

2.1.3. Diets

A standard rat maintenance diet was prepared, without the full complement of soybean meal or oil, by Ke-Ao-Xie-Li Feed Company. Three dietary incorporation levels, 7.5%, 15%, or 30%, were prepared for the CV127 and Conquista (control) diets. The nutrient composition of the experimental diets was determined in accordance with Chinese Standard (Chinese Standard GB/T5009.10–2003, 2003; Chinese Standard GB/T5009.6–2003, 2003; Chinese Standard GB/T5009.5–2003, 2003; Chinese Standard GB/T5009.4–2003, 2003; Chinese Standard GB/T5009.3–2003, 2003) These Chinese standards are methodologically equivalent to analogous OECD, EPA and other international approaches. All diets were vacuum-packed with polyethylene bags and sterilized with ^{60}Co radiation.

All diets were evaluated for the presence of the soybean reference gene *lectin* and an event-specific gene by PCR. The *lectin* gene was detected in all samples, while the event-specific gene was detected only in CV127 diets, thereby verifying the identity of the diets. Mycotoxins and pesticides were either not detected or detected below the minimum residue levels established for each pesticide.

2.1.4. Experimental design

Animals were distributed according to weight among the individual test groups ($n = 7$) with 20 rats in each group (ten male and ten female) for a total of 140 rats. A standard rat maintenance diet served as an additional control group. Animals were separated into treatment groups by randomization such that the mean body weight across groups did not vary by more than 20%. Each soybean meal treatment was administered via dietary incorporation over a 90-day period.