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Genetically Modified Feeds in Poultry Diet: Safety, Performance, and Product Quality

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Concerns have been expressed regarding the safety of using biotechnology derived feeds in diets of livestock animals and in regard to human consumption of products from species fed transgenic crops. As a consequence, a large number of poultry nutrition studies have been conducted to evaluate the wholesomeness of transgenic crops by examining performances of animals during growth or egg laying. Studies also evaluated whether foreign DNA and proteins could be detected in meat, egg, and tissue samples from broiler chickens and laying hens fed diets containing transgenic feeds. In all studies, the conclusions were in agreement that the transgenic crops provided comparable performance, carcass and egg yields, and meat and egg composition, when compared with conventional grains. Moreover, it was demonstrated that transgenic proteins and DNA present in livestock feeds are not detectable in food products derived from these animals, using the most sensitive detection methods available, confirming that they are rapidly degraded by normal digestive processes. The lack of significant differences were a result of the similarity in nutrient composition of the genetically modified feeds and lack of differences in intake and digestibility, while there were no evidences that the differences reported for performance response variables and carcass measurements between treatment groups were attributable to the presence of the transgenic gene and protein in the biotechnology derived plants. Results demonstrated that genetically modified feeds are substantially equivalent and they result as safe as existing conventional feeds.

Keywords Genetically modified feeds, poultry, performance, safety

INTRODUCTION

In a very short period of time, advanced biotechnologies, based on recombinant DNA technology, have permitted to produce a great deal of new crop varieties, commonly referred to as GM plants. The largest applications of genetic engineering in agriculture have concentrated on the high-volume crops: corn, soybean, cotton, and canola. The GM plants to provide herbicide tolerance were developed through biotechnology to contain the gene that make them resistant either to the active ingredients in commonly used agricultural herbicides, glyphosate (Roundup[®]) in so-called Roundup Ready[®] (RR) seeds or glyphosate in LibertyLink[®] seeds.

Glyphosate, one of the most extensively used herbicides in the world, is a nonselective systemic herbicide that is highly

effective against a broad spectrum of both annual and perennial grasses as well as broadleaf weeds (Malik et al., 1989). The herbicide inhibits the function of 5-enolpyruvylshikimate-3-phosphate synthases (EPSPS), an enzyme involved in the shikimic acid pathway for the biosynthesis of essential aromatic amino acids (phenylalanine, tyrosine, tryptophan) in plants (Steinruken and Amrhein, 1980). EPSPS is only present in plants and microorganisms, and it is absent in animals and humans (Levin and Sprinsor, 1964; Steinrucken and Amrhein, 1980). Glyphosate is practically nontoxic to nonplant life forms such as aquatic and avian species, animals, and man (Malik et al., 1989). Because of its lack of selectivity, glyphosate cannot be used during the active growth season of the crop.

A large number of herbicide-tolerant crop varieties have been produced by the stable insertion of a gene that encodes a modified EPSPS protein, designated as CP4 EPSPS, which allows the plant to synthesize essential aromatic amino acids. The CP4 EPSPS coding sequence derives from *Agrobacterium* spp. strain CP4; it encodes a CP4 EPSPS enzyme functionally similar to plant EPSPS enzyme but having a much 51 reduced affinity for

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glyphosate. Expression of this modified protein in plants imparts high levels of glyphosate tolerance.

Some herbicide-tolerant plants were produced by the stable insertion of an additional gene, the *gox* gene, derived from the *Ochrobactrum anthropi* strain LBAA, a commonly occurring bacterium in the rhizosphere (Lebuhn et al., 2000), which encodes for the production of an glyphosate degrading enzyme, glyphosate oxidase enzyme (GOX). The GOX enzyme accelerates the normal degradation of glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate. Both CP4 EPSPS and GOX proteins therefore confer tolerance to the application of glyphosate herbicide.

Transgenic plants, which have been modified to provide tolerance to glufosinate ammonium herbicide, contain the phosphoenolpyruvate carboxylase (PAT) gene, derived from *Streptomyces hygroscopicus* or *Streptomyces viridochromogenes*. The *pat* gene encodes an enzyme that will break down the herbicide glufosinate (phosphinothricin).

Other plants have been developed through biotechnology to be insect protected and are commonly referred to as Bt hybrids. GM varieties express a gene that enable the plants to produce an insecticidal protein against pests such as the European corn borer (*Ostrinia nubilalis*) infestation (Koziel et al., 1993) in the case of corn plants, or against the boll weevil in the case of cotton plants.

Numerous different insect-resistant varieties have been developed utilizing single Bt gene segments, and thereby expressing different and specific proteins including Cry1Ab, Cry1Ac, Cry1C, Cry1F, Cry2A, Cry3B, and Cry9C. The Cry gene derived from *Bacillus thuringiensis*, a natural occurring soil bacterium, which encodes for the production of a crystalline protein inclusion (Cry) in the form of protoxin that controls insects by disrupting the insect's digestive system. When consumed by the larval stage of the European corn borer and other lepidopteran insects, following a single acute exposure, the Cry protein is activated. Toxin binds to specific receptors in the mid gut cells and creates ion-selective channels in the cell membrane (English and Slatin, 1992), thus disturbing the osmotic balance and causing cellular swelling due to an influx of water which leads to the lysis of epithelial cells, as a result of which larvae stop feeding and eventually die (Knowles and Ellar, 1987). Only insects that are susceptible to the Bt protein have these receptors (English and Slatin, 1992). This protein has been found to be harmless to humans, fish, wildlife, and beneficial insects. Both herbicide-tolerance and insect-protection genes have been introduced into crops to provide weed and insect control. The combined trait crops were produced by conventional breeding of two transgenically derived single-trait products, Bt and RR crops.

The Principle of Substantial Equivalence for the Safety Evaluation of Biotechnology Derived Foods

As a consequence of the rapid increase in plantings of transgenic crops, concerns have been raised about human and animal health risks that might result by the use of these biotechnology

derived foods and feeds. The safety assessment of GM crops has attracted the attention of plant breeders, consumers, scientists, risk assessors, and regulators. A most important concern is whether safety evaluation of foods derived from crops produced through biotechnology based on the *substantial equivalence* concept is appropriate or new safety assessment approaches for the risk assessment are needed. The principle of substantial equivalence for the safety evaluation of biotechnology derived foods was approved and utilized by many leading international food organizations and regulatory bodies including the World Health Organization (WHO, 1991; WHO, 1995), the Organization for Economic Cooperation and Development (OECD, 1993; OECD, 1996), the Food and Agriculture Organization of the United Nations (FAO, 1996), and the International Life Sciences Institute (ILSI, 1997). These regulatory agencies have concluded that foods and feeds derived from GM crops, which have been approved, are as safe as the ones derived from conventional plants.

The European Union has adopted substantial equivalence method as part of the basis for the safety assessment of foods derived from crops developed through biotechnology and has approved several products based on this approach. In 1990, the European Commission has amended the first regulatory system, the European Council Directive 90/220/EC of 23 April 1990; successively, it was repealed by the new Directive 2001/18/EC concerning the deliberate release into the environment of GM organisms, which required an evaluation of risks for human and animal health and environment before GM organisms could be imported in Europe. The recently amended Regulations 1829/2003/EC and 1830/2003/EC of the European Parliament and of the Council of 22 September 2003 laid down Community procedures for the authorization and supervision, and provisions for the traceability and labeling of food and feed products produced from transgenic crops. Authorized GM foods and feeds have been previously undergone a severe safety assessment through Community procedures before being placed on the market within the Community.

Digestive Fate of Transgenic DNA and Proteins

Concerns have been expressed regarding the safety of using biotechnology derived feeds in diets of food-producing animals and in regard to human consumption of animal products from farm animals fed transgenic crops. Questions concern the likelihood that transgenic DNA and protein could be transferred to and accumulate in the food products (milk, meat, and eggs) of animals fed derived from GM crops, and the likelihood that the consumption of such animal products could lead to adverse human health effects.

The DNA from biotech crops is made up of the same building blocks and it is broken down just like any other food and feed-stuffs DNA; besides the amount of biotech DNA in transgenic food is extremely small compared to the total plant DNA consumed by humans or farm animals. The United Nations (UN)

FAO and WHO (FAO/WHO, 1991) and the US FDA (FDA, 1992) have each stated that the consumption of DNA from all sources, including GM crops, is safe.

The food ingested is mechanically disrupted and the digestive secretions, primarily enzymes and acidity, process transgenic DNA, just as all other DNA; more than 85% of the plant DNA consumed by ruminant species is cleaved into smaller DNA fragments, and single or smaller constituents of nucleotides before entering the duodenum (McAllan, 1982). Nucleotides are typically deaminated prior to being rapidly absorbed; once absorbed, they are further catabolized into nitrogenous bases, free bases, and secondary metabolites (McAllan, 1980). However, it is suggested that any small proportion of plant DNA fragments remaining in intestinal digesta that might enter the body would be phagocytized by tissue macrophages, dendritic cells, or other terminally differentiated phagocytes of the immune system (Beever and Kemp, 2000).

Allergenicity is one of the major concerns about food derived from transgenic crops. The digestive fate of each expressed protein in transgenic crops has been assessed on a case-by-case basis by *in vitro* digestive studies as part of the evaluation of the allergenic potential of the new proteins (Harrison et al., 1996; Metcalf et al., 1996). The safety of most Bt toxins is assured by their easy digestibility as well as by their lack of intrinsic activity in mammalian systems (Betz et al., 2000; Siegel, 2001). Ingested proteins introduced into GM products have been shown to be hydrolyzed into increasingly smaller fragments in the digestive system (Metcalf et al., 1996; Betz et al., 2000) and absorbed as small peptides or free amino acids, just as all other feed proteins.

Biotechnology Derived Foods as Feedstuffs for Livestock

Biotechnology derived crops which may be used in animal feeding have been subjected to animal wholesomeness studies to detect possible negative effects of diets containing products derived from transgenic plant on performance and health of livestock. Testing of GM feeds on laboratory animals has its specific problems, because of the limited quantity of feed that can be administered to animals compared with the amount for human or other animal diets. Hence, feeding experiments were carried out with broilers, layers, pigs, sheep, steers, and dairy cows to compare transgenic crops to conventional crops. The main objectives of these studies were to establish that the biotechnology derived feeds are nutritionally equivalent to conventional feeds and that they are as safe to animals and humans (Swiatkiewicz and Arczewska-Włosek, 2011).

A broad array of poultry nutritional studies has been conducted. Broilers were chosen as a useful model in a large number of research because of their rapid growth period. Reduced growth and carcass yields, and changes in meat composition would be a direct effect of deficiency or reduced bioavailability of nutrients in the diet because of their sensitivity to changes in nutrient quality.

To assess nutritional bioequivalency and feed safety of biotech crops, comparison of the agronomic characteristics and nutritional composition of the new crops to their conventional counterparts were conducted (Swiatkiewicz and Arczewska-Włosek, 2011). It is essential for GM feeds and foods safety assessment to verify any significant changes in the nutritional profile of the crops derived from the insertion of the transgene or its derived protein.

Previous trials investigated nutritional composition (main nutrients as crude protein, ether extract, crude fiber, crude ash, amino-acids, fatty acids, minerals, fiber components, and some mycotoxins) of the modified feed, levels of potentially toxic components, digestibility of nutrients, and the wholesomeness of transgenic crops by examining possible influence on animal health and product quality and the presence of the recombinant DNA and of the new protein.

Studies on the Nutritional Equivalence and Digestibility of GM Crops

In order to obtain significant comparative data from animal feed studies, particular attention was paid to the choice of experimental design, comparators (GM plant and nontransgenic control) were grown under the same environmental conditions, as environmental conditions may lead to compositional differences), number of animals, and statistical analysis of collected data. As a result, the studies provided an opportunity to evaluate any unintended effect that may not have been detected by the other methods to support a conclusion of substantial equivalence (Swiatkiewicz and Arczewska-Włosek, 2011).

A great deal of studies have been carried out to evaluate the compositional profile of biotech crops compared with that of nontransgenic controls as well as with those of traditional hybrids available commercially. The feeding value of both herbicide-tolerant and insect protected crop varieties was found to be comparable to that of non-GM varieties. The values for the biochemical components assessed for glyphosate-tolerant corn (Sidhu et al., 2000; Ridley et al., 2002), soybeans (177; Hammond et al., 1996; Padgett et al., 1996; Taylor et al., 1999), wheat (Obert et al., 2004), and cotton (Nida et al., 1996) were equivalent to those of nontransgenic parent as well as to conventional varieties or they were within the published range observed for nontransgenic commercial crop hybrids. Compositional equivalence assessment has been also reported for insect protected crops including soybean (Kan and Hartnell, 2004) and corn (Brake and Vlachos, 1998; Halle et al., 1998; Mireles et al., 2000; Aulrich et al., 2001).

Digestion and feeding experiments were carried out by Flackowsky and Aulrich (2001) with broilers (insect-resistant maize) and layers (insect-resistant maize, glufosinate-tolerant maize). No differences were observed between birds fed diets containing GM or not-GM feeds. The study of Aulrich et al. (1998) showed that layers had the same digestibility of organic matter, protein, and metabolizable energy, whether they were fed

Bt corn or non-Bt corn diets. No differences in apparent metabolizable digestibility coefficients (Gaines et al., 2001), protein digestibility (Halle et al., 1998), true metabolizable energy, and amino acid digestibility (Mireles et al., 2000) were observed in broiler chickens fed biotech corns compared with birds fed their near isogenetic parents in diets. The observed minor differences in proximate values may have been related to differences in crop density or moisture content, which are known to occur across hybrids and growing conditions, and to general health of the plant (Brake and Vlachos, 1998).

The results of compositional analyses generated from these studies demonstrated that the grain and forage of biotech crops are comparable in their composition with those of the nontransgenic control and conventional crop varieties. These comparisons support the conclusion that biotech crops are compositionally equivalent to, and nutritious as, conventional crops.

Studies on the Transfer of Transgenic DNA and Protein Fragments from Feeds into the Poultry Tissues

As questions about the digestive process and metabolic fate of transgenic DNA and proteins have been raised, several studies have been conducted to evaluate whether foreign DNA and proteins can be detected in meat, egg, and tissue samples from broiler chickens and laying hens fed diets containing transgenic foods. Supporting references showed that neither transgenic DNA nor protein fragments have been detected in meat and eggs produced from birds receiving GM feed ingredients using the most sensitive detection methods available (Aeschbacher et al., 2001; Khumnirdetch et al., 2001; Glenn, 2001). Utilizing an enzyme-linked immunosorbent assay (ELISA) test, Ash et al. (2000) did not find CP4 EPSPS protein in liver, feces, whole egg, and egg white from laying hens fed RR soybeans. Neither using highly sensitive polymerase chain reaction (PCR) amplification techniques coupled to Southern blot detection of specific amplicons, small fragments of the *CryIAb* and *sh2* genes nor using a sensitive competitive ELISA, intact or immunoreactive fragments of the *CryIAb* protein were detected in breast meat samples from chickens fed for 42 days a diet highly enriched in YieldGard Corn Borer corn (Jennings et al., 2003). A Japanese government study also failed to detect *Cry9C* protein in samples of muscle, liver, or blood from chickens fed a diet containing StarLink® maize (Japan MAFF, 2001).

In a study, Rasche (1998) investigated the stability of transgenic DNA encoding the phosphinothricin N214 acetyltransferase enzyme from herbicide-tolerant canola in digestive fluids isolated from chickens. It was observed that the DNA was completely degraded to nucleotides within one hour at body temperature and pH 1.5. Einspanier and co-workers (2001) investigated the fate of ingested transgenic plant DNA in chickens being fed a diet containing conventional maize or insect-resistant maize. The study was unable to detect even small fragments of *CryIAb* encoding genes in any sample (muscle, liver, spleen, and kidney) from poultry; in contrast, short DNA fragments (<200 bp)

of plant chloroplasts (non-GM) were detected in all chicken tissues.

In a review of the safety issues associated with DNA in animal feedstuffs derived from GM crops, Beever and Kemp (2000) provided comprehensive information of the processes of DNA digestion in both ruminants and nonruminants and concluded that under normal circumstances there appeared to be little opportunity for intact plant DNA to be absorbed from the gastrointestinal tract. These studies demonstrated that transgenic proteins and DNA present in livestock feeds are not detectable in food products derived from these animals, confirming that they are rapidly degraded by normal digestive processes.

Studies on the Feeding value of Insect-Protected Crops for Poultry

Many feeding studies have been conducted to evaluate possible unexpected nutritional effects of diets containing insect-resistant or herbicide tolerant feeds on broiler growth, egg production, and performance, impacting the production economics (Swiatkiewicz and Arczewska-Włosek, 2011). All the experimental design consisted of diets made from different types of biotechnology derived ingredients and formulated based on the individual nutrient analyses of these ingredients of each genotype; moreover, analysis of poultry diets were conducted to confirm formulated nutrient composition.

Performances and carcass yields of broilers were compared when fed insect-resistant crops, nontransgenic parental control line and commercial varieties and no differences attributed to crop source were detected. Broilers fed different corn varieties had similar body weight gain, feed intake, feed conversion, and carcass composition (Halle et al., 1998; Mireles et al., 2000). Brake et al. (2003) evaluated whether diets containing grain from the Bt-corn either sprayed with glufosinate or nonsprayed, and nontransgenic corn had any adverse effect on male and female broiler chickens performances. Body weight of broilers was excellent and there were no differences in feed conversion ratio, survivability, and percentage carcass yield due to corn source to 42 days. The study by Aulrich et al. (2001) also concerned comparisons between an insect-resistant maize variety and its non-GM equivalent fed to poultry. Again, no differences were found in body weight between birds on the GM diet and those on the non-GM diet.

Poultry feeding studies have also been conducted with solvent extract dehulled soybean meal from transgenic Bt soybeans (Kan and Hartnell, 2004). Final live weight, feed conversion, carcass yield, and breast meat composition of broilers fed the transgenic soybean meal were identical to birds fed nontransgenic soybean meal. The introduction of Bt gene resulted in a soybean meal that was nutritionally equivalent to the parental control or commercial varieties. Differently, in the experiment of Brake and Vlachos (1998) broiler chickens fed mash or pelleted diets prepared with transgenic event 176 Bt corn had similar body weight, but birds exhibited a significant improvement in

feed conversion ratio and breast meat yield as compared with birds receiving diets prepared with the corresponding conventional corn. However, authors concluded that differences were not attributable to the transgenic corn *per se*. In the experiment of Piva et al. (2001), there were nondifferences in feed intake, average daily gain, and feed/gain between broilers fed insect protected corn or its near isogenic control corn, but final live weight was significantly greater for the animals fed the Bt diet. They concluded that differences were due to a lower level of fumonisin B1 in Bt corn than in control corn.

Feeding diets that contained Bt maize to broiler chickens resulted in comparable weight gain values, but significantly decreased average daily feed intake and tended to increase feed/gain compared with its near isogenetic parent and commercial corn hybrids (Gaines et al., 2001). Leeson (1998) reported an increased mortality of male broilers fed with GM corn.

In later studies, broilers fed diets containing insect-protected corn (Taylor et al., 2001a; Taylor et al., 2002b; Taylor et al., 2003b; Taylor et al., 2003c) overall had similar performance parameters, carcass yields, and meat compositions as compared with their respective nontransgenic controls or commercial reference corns. Differences between treatment groups were reported for breast meat, thigh, drum, and wing weights (Taylor et al., 2003c), and for breast meat and drumstick weights (Taylor et al., 2003b).

In the cases for each experiment in which differences were observed, the variability in performance data between birds receiving the transgenic corn diets and those that received the corresponding conventional corn hybrids did not exceed the variation reported in literature values. It was clear that the transgenic corns and soybeans had no deleterious effects on production traits of broiler chickens. Results indicate that performance of poultry is as least as good when birds are fed insect-protected and non-Bt crops.

Studies on the Feeding value of Herbicide-Tolerant Crops for Poultry

Poultry feeding studies were conducted to compare the feeding value of herbicide-tolerant crops fed to broiler chickens and laying hens to the parental line. Nutritional value of grain from glyphosate-tolerant maize was evaluated in a poultry feeding study by Sidhu et al. (2000). Results showed that there were no differences in growth, feed efficiency, and fat pad weight between chickens fed diets containing glyphosate-tolerant corn and diets containing conventional corn. Weight gain, feed conversion, breast meat yield, and fat pad weight were similar for broilers fed both transgenic and conventional soybean lines (Hammond et al., 1996). Performance parameters (weight gain, feed intake, and feed gain ratio) were not different for broilers fed glyphosate tolerant corn and control diets (Gaines et al., 2001). Recently, new experiments were conducted to compare the nutritional value of RR corn (Taylor et al., 2001b; Taylor et al., 2003a) and RR canola meal (Taylor et al., 2004) to their

conventional counterparts by measuring performance parameters, carcass yield and meat composition parameters in broilers. Broilers fed diets containing glyphosate-tolerant crops had similar growth performance to birds fed nontransgenic control and commercial diets. Some differences between treatment groups were noted for breast meat and fat pad weights, and percentage moisture content of the thigh meat (Taylor et al., 2003a), feed intake, final live weight, adjusted feed conversion, and chill weight (Taylor et al., 2004), but these differences were considered consistent with natural variability. The results of these studies indicated that Roundup Ready crops containing the CP4 EPSPS protein were nutritionally equivalent to their corresponding parental line and commercial lines when fed to broilers.

Studies on the Feeding value of Insect Protected × Herbicide Tolerant Crops for Poultry

Both herbicide-tolerance and insect-protection traits have been introduced into corn and soybean to provide efficacious, environmentally compatible methods of weed and insect control. Previous experiments compared the nutritional value of YieldGard × RR corn (Taylor et al. 2001a; Taylor et al., 2002a; Taylor et al., 2003a; Taylor et al., 2003b) to their respective nontransgenic controls and commercial corn. In these studies, growth performance, feed conversion, and meat composition have been evaluated with no observable differences. On the contrary, differences between treatment groups were described for protein content of breast meat (Taylor et al., 2003a) and for breast meat and drum weights (Taylor et al., 2003b), but these differences were considered to be irrelevant biologically. In conclusion, there were no relevant differences in terms of feeding value, performance, carcass yield, meat, and eggs composition between the transgenic feedstuffs and their respective nontransgenic controls when fed to chicken broilers and laying hens. No evidence of animal health problems associated specifically with the ingestion of these crops or resulting food products have been identified.

Studies on the Feeding value of Crops that have Increased Nutritive value for Poultry

The GM varieties of plants that have traits to enhance nutritional properties are called second-generation crops. Genetic engineering led to enhanced availability of phosphorus, increased growth performance and phosphorus digestibility, and decreased excretion of phosphorus in broilers fed low nonphytate phosphorus diets containing soybeans transformed with a fungal phytase gene (Denbow et al., 1998). The nutritional value of broiler feeds was enhanced through recombinant techniques, resulting in improved daily gain and feed efficiency, and number of chickens with adhering sticky droppings drastically reduced (Von Wettstein et al., 2000). Protein concentration, energy and sulfur

amino acids digestibility were higher in soybean meal processed from GM high-protein soybeans, resulting in considerable advantages over conventional soybean meal as a feed ingredient for broiler chickens because of the corn soybean meal diets are first-limiting in sulfur amino acids (Edwards et al., 2000). Moreover, biotech crops may enhance safety for foods and feeds, and animal products, as levels of mycotoxins (fumonisins) for corn grain were lower for insect protected varieties than for their nontransgenic control lines (Munkvold et al., 1999; Piva et al., 2001).

CONCLUSIONS

Results provide further assurance that the current GM crops are substantially equivalent to and safe as their traditional counterparts in composition, digestibility, nutritional value for livestock, and they provide comparable performances and carcass yields when compared with the conventional counterparts (Clark and Ipharraguerre, 2001). Moreover, plant DNA and proteins introduced into biotech crops approved for food and feed consumption have not been detected in animal products from laying hens and broiler chickens using the most sensitive detection methods available. For those commercially available GM crops that are components of livestock feeds, there is no evidence of significantly altered nutritional composition, deleterious effects, or the occurrence of transgenic DNA or proteins in foods of animal origin. Studies of this type have established that the level of safety to animals of genetically engineered feedstuffs is likely to be equivalent to that of traditional feeds, although the current safety evaluation system probably would not detect minor or rare adverse effects. Actually, none of these GM foods has been subjected to a long-term study of its effects on health.

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