Multiple organ histopathological changes in broiler chickens fed on genetically modified organism

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Abstract
Diet can influence the structural characteristics of internal organs. An experiment involving 130 meat broilers was conducted during 42 days (life term for a meat broiler) to study the effect of feed with protein from genetically modified soy. The 1-day-old birds were randomly allocated to five study groups, fed with soy, sunflower, wheat, fish flour, PC starter. In the diet of each group, an amount of protein from soy was replaced with genetically modified soy (I – 0%, II – 25%, III – 50%, IV – 75%, V – 100% protein from genetically modified soy). The level of protein in soy, either modified, or non-modified, was the same. Organs and carcass weights were measured at about 42 days of age of the birds and histopathology exams were performed during May–June 2009. No statistically significant differences were observed in mortality, growth performance variables or carcass and organ yields between broilers consuming diets produced with genetically modified soybean fractions and those consuming diets produced with near-isoline control soybean fractions. Inflammatory and degenerative liver lesions, muscle hypertrophy, hemorrhagic necrosis of bursa, kidney focal tubular necrosis, necrosis and superficial ulceration of bowel and pancreatic dystrophies were found in tissues from broilers fed on protein from genetically modified soy. Different types of lesions found in our study might be due to other causes (parasites, viral) superimposed but their presence exclusively in groups fed with modified soy raises some serious questions about the consequences of use of this type of feed.

Keywords: genetically modified soy, liver histopathology changes, inflammation.

Introduction
Transformation event soybean (Glycine max) GTS 40–2–3, sold as Roundup Ready (RR) was created for the use of glyphosate-based herbicides for total weed control in commercial crops. The simplicity of this system was one reason for the quick success among farmers (AGBIOS, 2009). GTS 40–2–3 was obtained by introducing the EPSPS gene isolated from the line CP4 of the species Agrobacterium tumefaciens in the commercial variety A5403 (Asgrow Seed Company). The introduced EPSPS gene encodes an insensitive protein, in general, of the action of glyphosate, which can thus metabolize aromatic amino acids necessary to the plant, even when applying the herbicide Roundup.

In Romania, since 2007, the cultivation of genetically modified soybeans is prohibited, but not its import. In this way, each year large quantities of soybeans and soybean meal used in feed intended for human consumption or as an ingredient in preparations of feed and food enter the country. The legislation requires the producers to inform consumers by labeling the products, which use as an ingredient of a genetically modified organism. There is no reference to the use of genetically modified organisms in feeding animals for human consumption.

Food safety and animal genetic modified food is a subject of debate since the first marketing of the product (1970) and there seems to be no clear direction. The safety assessment of genetically modified (GM) plants and derived food and feed follows a comparative approach; food and feed are compared with their non-GM counterparts to identify their undesirable effects. The differences are then evaluated in terms of their potential impact on the environment, safety for humans and animals and also in terms of nutritional quality. The key elements of the assessment procedure are the molecular, compositional, phenotypic and agronomic analysis, in order to identify similarities and differences between the GM plant and its isogenic counterpart [1]. The safety assessment focuses on the presence and characteristics of newly expressed proteins and other new components and possible changes in the level of natural constituents beyond normal variation, the possible occurrence of unanticipated effects from genetically modified plants through genetic modifi-
cation. To identify these effects, a comparative phenotypic and molecular analysis of genetically modified plants and their counterpart parallel isogenic are carried out. In the same time, specific analysis of specific compounds is performed, such as macro and micro-nutrients, known anti nutrients and toxins. An important aspect is the potential impact of transgenic animal feed on animal metabolism.

Materials and Methods

Our objective was identifying the effects of genetically modified food on the broiler with possible public health implications, taking into consideration the importance of chicken in contemporary human nutrition. The emergence of significant differences may be indicative of the occurrence of adverse effects, which require further investigation.

As study animals, Ross commercial broiler were used, breed intensively on permanent litter. The study was focused on a number of randomly selected 130 chickens from a batch of 14 000 one day old chicks, using as selection criteria health, weight and sex. The 130 subjects, weighing 40–42 g, sex ratio 1:1 were divided into five groups. In the diet of each group, an amount of protein from soy was replaced with genetically modified soy line GTS 40–2–3 in a following proportion: 0 – group I; 25% – group II, 50% – group III; 75% – group IV, 100% – group V. Table 1 presents components of feed used in the three stages of growth for each group of study.

Table 1 – Components of feed used in the three stages of growth (starter – S, growth – G, and finishing – F)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>S</td>
<td>G</td>
<td>F</td>
<td>S</td>
<td>G</td>
</tr>
<tr>
<td>Corn</td>
<td>454</td>
<td>505</td>
<td>510</td>
<td>454</td>
<td>505</td>
</tr>
<tr>
<td>Wheat</td>
<td>100</td>
<td>100</td>
<td>150</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Conventional soy (heat treated)</td>
<td>330</td>
<td>290</td>
<td>264</td>
<td>247.5</td>
<td>217.5</td>
</tr>
<tr>
<td>Modified soy (heat treated)</td>
<td>0</td>
<td>0</td>
<td>82.5</td>
<td>72.5</td>
<td>66</td>
</tr>
<tr>
<td>Fish flower</td>
<td>550</td>
<td>30</td>
<td>550</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Sunflower hull</td>
<td>26</td>
<td>35</td>
<td>36</td>
<td>26</td>
<td>35</td>
</tr>
<tr>
<td>PC</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 1 – Components of feed used in the three stages of growth (starter – S, growth – G, and finishing – F)

After 42 days, the subjects were sacrificed by cervical dislocation and necropsy was done after careful weighing of carcasses, parts of carcasses and internal organs. Sampling of the small intestine, muscle, liver, heart, stock and kidneys as anatomical parts was taken in order to carry out histo logical tests. For histology and histochemistry at light microscopy, samples were fixed with 4% neutral buffered formalin and embedded in paraffin wax. Five mm-thick cross sections of samples were either stained with Hematoxylin–Eosin (HE) or Masson’s trichromic (TM) method for morphological examinations.

Results

Total weights and detailed anatomical parts and the viscera, the offspring of the five groups are presented in Table 2.

Table 2 – Weight [g] of the carcasses and viscera of some somatic areas

<table>
<thead>
<tr>
<th>Group</th>
<th>Carcasses</th>
<th>Wings</th>
<th>Legs</th>
<th>Chest</th>
<th>Heart</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1930 (116.1)</td>
<td>89.5 (7)</td>
<td>229.3 (11.5)</td>
<td>486.5 (12.1)</td>
<td>9.5 (1.4)</td>
<td>54 (4)</td>
</tr>
<tr>
<td>Group II</td>
<td>1953 (115)</td>
<td>95.5 (8)</td>
<td>232.6 (11.2)</td>
<td>515.1 (11.3)</td>
<td>10 (1.9)</td>
<td>52 (5.5)</td>
</tr>
<tr>
<td>Group III</td>
<td>1740 (111)</td>
<td>85.3 (7)</td>
<td>205.2 (12)</td>
<td>470.2 (13)</td>
<td>10 (1.9)</td>
<td>46 (5.6)</td>
</tr>
<tr>
<td>Group IV</td>
<td>1832.6 (113.2)</td>
<td>92.6 (7)</td>
<td>211.2 (11)</td>
<td>445.3 (11.5)</td>
<td>9.5 (2)</td>
<td>48 (4.2)</td>
</tr>
<tr>
<td>Group V</td>
<td>2297 (115)</td>
<td>114.2 (7.2)</td>
<td>255.8 (12)</td>
<td>510 (9.5)</td>
<td>9.5 (1.9)</td>
<td>50 (5)</td>
</tr>
</tbody>
</table>

One can see that there are some weight differences; the subjects from the fifth chickens group have the higher weight figures. The ANOVA method, using processed raw data, did not identify significant effects of genetically modified soy consumption on weight carcasses – F(4.25)=1.89, p>0.05 – weighed of the carcass parts: F(4.25)=2.4, p>0.05 – for wings, F(4.25)=1.89, p>0.05 – for legs, F(4.25)=1.53, p>0.05 – for chest or heart weight: F(4.25)=0.16, p>0.05 – for heart.

Regarding the liver, significant differences between groups were found [F(4.25)=5.2, p=0.003]. Post-hoc tests indicate that liver weight in chickens group V is significantly higher than that of chickens in groups I and II, but not than the chickens in groups III and IV (p<0.05).

Histopathology lesions are found over the liver, intestine, pancreas, kidney and striated muscle’s attestation even in subjects coming from group II. Obvious changes on these levels are found in subjects from groups IV (75% GM soy) and V (100% GMO soy). The following changes have been found in the liver tissue in group II subjects: hepatocytes with vesicular nuclei, enlarged sinusoids and reduced portal lymphoplasmocytar infiltrate. In group III, small areas stand peliosis liver, intralobular inflammatory granulomas and incipient porto-portal fibrosis (Figure 1 A, B). Lesions of interface hepatitis are added to the subjects in group IV (Figure 1). Tissues from subjects in group V present steatosis lesions, small deposits of glycogen, portal and intralobular lymphocytes groups, dilated vein and hepatocytes with small irregularly
shaped nuclei. Focal necrobiosis and necrosis, anucleate hepatocyte and even proliferating bile ducts are also encountered (Figure 1).

The bursa of Fabricius, we notice the presence of a whitish film and some superficial ulcerations or focal hemorrhage (Figure 2).

The intestine tissue shows necrotic lesions or ulcers in the superficial causal bags on colon. On the subjects in group IV and V hyperplasia of mucosal intestine, conjunctive and vascular polyps, granulocytic and lymphocytes dissecting inflammation were observed (Figure 3).

Lesions identified in the renal tissue on subjects fed on GMO protein are limited to focal tubular necrosis (Figure 4).

Striated muscle tissue taken from the chest and leg derived from subjects food which were introduced genetically modified organisms show hypertrophy and fragmentation of muscle fibers (Figure 5).

Figure 1 – Histopathology changes present in the liver in subjects coming from groups III, IV and V: (A) Group III subject: hepatitis interface, intralobular lymphocytic infiltrate (Masson’s trichromic stain, 2×50%); (B) Group IV subject: intralobular inflammatory granuloma, enlarged sinusoids, peliosis (Hematoxylin–Eosin stain, 4×50%); (C) Group IV subject: biliary neocanalici, disruption of the lobular structures, porta-centrolobulary bridge, plasmocytes and lymphocytes infiltration (Masson’s trichromic stain, 2×50%); (D) Group IV subject: fibrosis and proliferation of biliary neocanalici (Masson’s trichromic stain, 4×75%); (E) Group V subject: interface hepatitis, peliosis, fibrosis (Masson’s trichromic stain, 2×100%); (F) Group V subject: lesions of biliary canalici, periportal hepatocyte necrosis (Green Masson’s trichromic stain, 4×100%).

Figure 2 – Histopathology changes present in the bursa of Fabricius in subjects coming from groups III, IV and V: (A) Group III subject: superficial ulcerations, necrotic and inflammatory areas (Masson’s trichromic stain, 2×50%); (B) Group IV subject: superficial erosions mucosal and inflammatory infiltrate in the lumen (Masson’s trichromic stain, 2×100%); (C) Group V subject: inflammation, superficial ulceration and necrotic material (Masson’s trichromic stain, 2×100%).
Discussion

Our study has identified changes on the subjects whose food contained protein MG on the following levels:

- the liver presents lesions of fibrosis, peliosis, necrosis, steatosis, bile ducts proliferation;
- the bursa of Fabricius presents white pellicle, some superficial ulceration and focal hemorrhage;
- the intestine presents mucosal hyperplasia, vascular polyps, joint inflammation and dissecting granulocytic and lymphoplasmocytic infiltration;
- striated muscle taken from the chest and legs...
present hypertrophy and fragmentation of muscle fibers. 

Variously modifications were identified in the nuclei of hepatocytes and microvilli of the mice fed on genetically modified soybean [2]. Other study showed reversibility of lesions after diet modification [3, 4]. Also, after 15 days of conventional soybeans administration on mice, zymogene depletion and acinar disorganization were found in the pancreas. These changes are also reversible within 30 days after diet change, excluding MG protein from the feed [5–7]. Changes in intestine microvilli and the caecal bags were observed on mice fed for 10 days with genetically modified raw potatoes. Lesions were not seen on subjects fed on the same potatoes but heat-treated [8]. According to other studies, the GM soybean-containing diet does not affect the gross anatomy or the histopathological features, even after a long lasting intake [9].

Different types of inflammatory lesions found in our study could be due to other additional causes (parasitic, viral and toxic) [10]. The natural variations in the composition of soybeans regarding natural or anthrop toxic substances, such as lectins or the concentration of phytoestrogens are to be discussed [11–14]. These differences are possible because of the GM protein derived from soybeans grown in Argentina in 2008 and the conventional production of soybeans grown in the Arad County in 2008. Comparative studies on the composition of GM soybeans and on the conventional soybeans indicate a lower level of phytoestrogens in unconventional soybean [15, 16]. This difference may explain some of the histological changes found if we consider the reduction of oxidative stress by soy isoflavones. Lectines effects should be mentioned. Soy natural glycoproteins Glycine max has a hemagglutinating effect on intestine epithelium and the liver tissue. A study on mice, into whose diet hemagglutinins was administering effect on intestine epithelium and the liver tissue.

Investigated the mechanism of action of these lectins in inflammatory and degenerative lesions in the liver, the subjects fed GM soy was introduced, revealed a significant difference between liver weight of carcass weight or its component parts but we can question, given the short period of feeding with these products, lack of long-term study on the offspring, the lack of a study of reproductive organs as well as fertilized eggs.

\section{Conclusions}

Introducing the genetically modified food in the broiler diets did not result in significant differences in terms of carcass weight or its component parts but we found a significant difference between liver weight of subjects fed on food genetically modified soybean protein 100% and those from non-GM soy group.

Histopathological examination of parts, from which the subjects fed GM soy was introduced, revealed inflammatory and degenerative lesions in the liver, inflammation and necrosis of the stock market, necrotic lesions and ulcers in the superficial caecal sacs, intestine mucosal hyperplasia, focal tubular necrosis, hypertrophy of some muscle fibers.

Different types of inflammatory lesions found in our study could be due to other additional causes (parasitic, viral, toxic), but their presence in subjects fed with genetically modified organisms raise serious questions about their use in feeding.

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