

HISTOPATHOLOGY OF INTERNAL ORGANS OF FARM ANIMALS FED GENETICALLY MODIFIED CORN AND SOYBEAN MEAL

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Abstract

Histopathological examination of liver, kidney, spleen, pancreas, duodenum, jejunum, skeletal muscle, and bursa of Fabricius samples, collected from broiler chickens, laying hens, fattening pigs, and calves fed genetically modified corn MON 810 and soybean meal MON-40-3-2 (Roundup Ready, RR), was performed. The examination showed no significant differences between the control animals fed diets containing no genetically modified feeds and animals fed genetically modified feeds. In some cases, congestion of parenchyma and focal lymphoid cell infiltrations were observed in all dietary groups, including controls, and therefore, it was assumed that the lesions were not associated with the feeding transgenic feeds.

Key words: farm animals, genetically modified feeds, histopathological analysis.

The issue of the use of feed materials derived from genetically modified (GM) crops in animal feeding raises a considerable controversy. Numerous reports on this topic have been published in the past with the conclusions on the lack of any harmful effects on animal and human health (2, 3, 5, 6, 14, 19), however, opposite opinions also exist and are experimentally documented (9, 15). Public concerns raised above all the possible impact of GM crops on the environment and food safety. Last but not least in the context of importance are also ethical, political, and economical questions. The concerns about the environment are mainly related to the cultivation of GM crops, and to a lesser extent to their later processing and use as feed. Cultivation of GM crops can have a potentially harmful impact on a number of non-target insect species (some of them beneficial to the agriculture or protected by law), as the modified proteins of the crops may have a negative effect on these species (4). Furthermore, the possible transfer of transgenic DNA to other organisms and its spread in the environment is taken under consideration (13). Concerns about food safety are associated with the possible subchronic toxicological effects of GM modified food components on physiological parameters of blood and organs in mammals and humans. Doubts are also raised by the theoretical possibility of absorption of new

transgenic DNA and derived proteins in the gastrointestinal tract and their transition into tissues and food products of animal origin (20). Discussion on these issues was intensive in recent months and resulted in preparing the new law on genetically modified organisms by the government, which will be probably voted on in the Polish parliament this year.

The aim of the present study was to investigate the impact of feeding with diets containing GM feeds on internal organs of farm animals by histopathological examination.

Material and Methods

All animals used in the experiment were raised as a part of research financed by the Ministry of Agriculture and Rural Development and performed at the National Research Institute of Animal Production with collaboration of National Veterinary Research Institute in Pulawy. All procedures included in the experiment, relating to the use of live animals, were conducted under the guidelines set forth by the Local Ethic Committee for Experimental Animals. During the whole study all animals were treated humanely and were under veterinary supervision.

GM feed materials. In the present experiment the most widespread genetically modified (GM) plant materials with the highest importance at the feed market (MON810 corn and MON-40-3-2, RR soybean meal) were used in the animal diets. Both of them were developed in agrotechnical traits and belong to the oldest type of plants' genetic modification, known as transgenesis of the first generation. RR soybean was developed by inserting a gene isolated from the *Agrobacterium* sp. CP4 strain of soil bacterium, expressing 5-enolpyruvylshikimate-3-phosphate synthase in plant. This enzyme confers a glyphosate tolerance upon plants. Glyphosate is an active ingredient in herbicide (Roundup). In case of corn, modification involved integration of gene isolated from *Bacillus thuringiensis* (*Bt*) in order to express the Cry1A(b) protein, which induces the resistance to the insect pest - European corn borer. A non-modified, isogenic parental line of corn (DKC 3420) and non-modified commercial soybean meal were used as controls.

Design of experiments. Feeding experiments were performed on broiler chickens, laying hens, fattening pigs, and calves. The content of nutrients and energy in all diets used in the study was consistent with the needs of animals and was similar in each of the feeding groups. The only factor differentiating the diets was the contribution of the genetically modified feed materials. The experimental setup of the experiment was the same for all but one (fattening pigs) animal species and was as follows: group I, control - a diet of grain corn and soybean meal from conventional (non-GM) lines, group II - corn line conventional and GM soybean meal (HT), group III - GM corn (*Bt*) and conventional soybean meal, group IV - corn and soybean meal from GM plants. In case of fatteners, two additional groups were created, *i.e.* group V feed a mixture containing unmodified soybean meal + cereal and group VI feed a mixture containing GM soybean meal + cereal. Correctness of preparation of experimental diets was confirmed by PCR analysis of specific transgenic DNA sequences of MON 810 maize and RR soybean.

For the study on broilers, 640 one-day-old Ross 308 chicks were obtained from commercial hatchery. Chickens were kept in pens (40 chickens each) set on a concrete floor covered with wood shavings, in an environmentally controlled room containing individual pen lamps and a ventilation system, with the constant access to feed and water. Four experimental groups (each consisting of four boxes) were created as described above. The experiment lasted 42 d.

In the study on laying hens, 96 Bovans Brown hens, between 25 and 54 weeks of age, were used. Hens were kept in individual cages measuring 40 x 35 cm (length/width), on a wire-mash floor, with constant access to feed and water. Each experimental group consisted of 24 birds kept individually in cages. The experiment lasted 30 weeks. The data concerning detailed methodology, as well as, broiler and hen performance and egg and meat quality results were already presented (17, 21, 22).

The study on pigs was carried out on 72 fatteners originated from (PL + LWP) sows mated with

boar (Du x Pi). Each group consisted of six gilts and six barrows. Pigs were kept in individual pens, and fed individually dosed amounts of feed depending on the body weight. Experimental fattening lasted from about 30 to about 110 kg of body weight and control weighing was performed every two weeks. At the end of the experiment, all pigs were slaughtered and samples of tissues were taken for histopathological examination. The detailed data concerning experimental methodology, as well as, fattening results and meat quality were already presented (16, 18).

The experiment on calves was carried out on 40 Polish Black-and-White HF bulls aged from 10 d at the beginning to 90 d at the end of the experiment. Calves were kept in individual cages (Alfa Laval) with slatted wooden floors covered with straw and equipped with automatic watering bowls and feeding troughs. The calves were divided into 4 groups of 10 animals each according to the analogue method based on live-weight, HF blood share (52%-87%), and calf condition. The calves were allocated into appropriate groups successively as they were born. The calves received liquid feed and concentrate. Before starting the experiment, the calves received only colostrum and whole milk. From the beginning of the trial to 56 d of age, all calves were fed liquid feed prepared from milk replacer powder (SanoRot). The calves were fed milk replacer solution according to IZ-PIB-INRA recommendations (7) formulating the protein and energy value of feeds, and proportion of ingredients in concentrates using INRation and PrévAlim version 3.x (2005) software. All concentrates were fed individually, *ad libitum* and contained similar amounts of corn (56%), soybean meal (25%), oat (15%), premix CJ Komplet (3%), and limestone (1%). The experimental groups of animals were fed the following concentrates: conventional line of corn and soybean meal (group I, control), conventional corn and modified soybean meal - HT (group II), modified corn (*Bt*) and conventional soybean meal (group III), modified corn (*Bt*) and modified soybean meal (HT) (group IV). Five calves from each group at the 90th d of age were slaughtered and samples of internal organs and skeletal muscles were taken for histopathological examination.

Sampling procedure and analysis.

Immediately after slaughter of the animals, their liver, kidneys, spleen, pancreas, duodenum, jejunum, and skeletal muscles were collected and fixed in 10% neutral buffered formalin. In case of broiler chickens, the bursa of Fabricius instead of muscles was collected. The number of animals from which samples were collected was as follows: broiler chickens and laying hens - 10 birds from each group (total 40 birds of each category), fatteners - six animals (three gilts and three hog) from each group (total 36 animals), calves - five animals from each group (total 20 animals). After fixation the tissue sections were processed by routine histological methods. Paraffin sections (5 µm) were stained with haematoxylin and eosin (HE) and examined under a Zeiss Axioskop 2 light microscope.

Statistical analysis. Statistical analysis was performed separately for broilers, laying hens, calves,

and pigs. The results were analysed using multiple comparison procedures (Kruskal-Wallis test, Dunnett's test, and Spearman rank correlation coefficients examination). For comparison of the fraction of positive results in the control groups with the results of other groups (GM soya, GM corn, GM corn + GM soya, control + cereals, GM soya + cereals), one-way analysis of variance ANOVA with Dunnett's test was applied. Significance level was set at 5%. The calculations were performed using STATISTICA software, version 10, StatSoft.

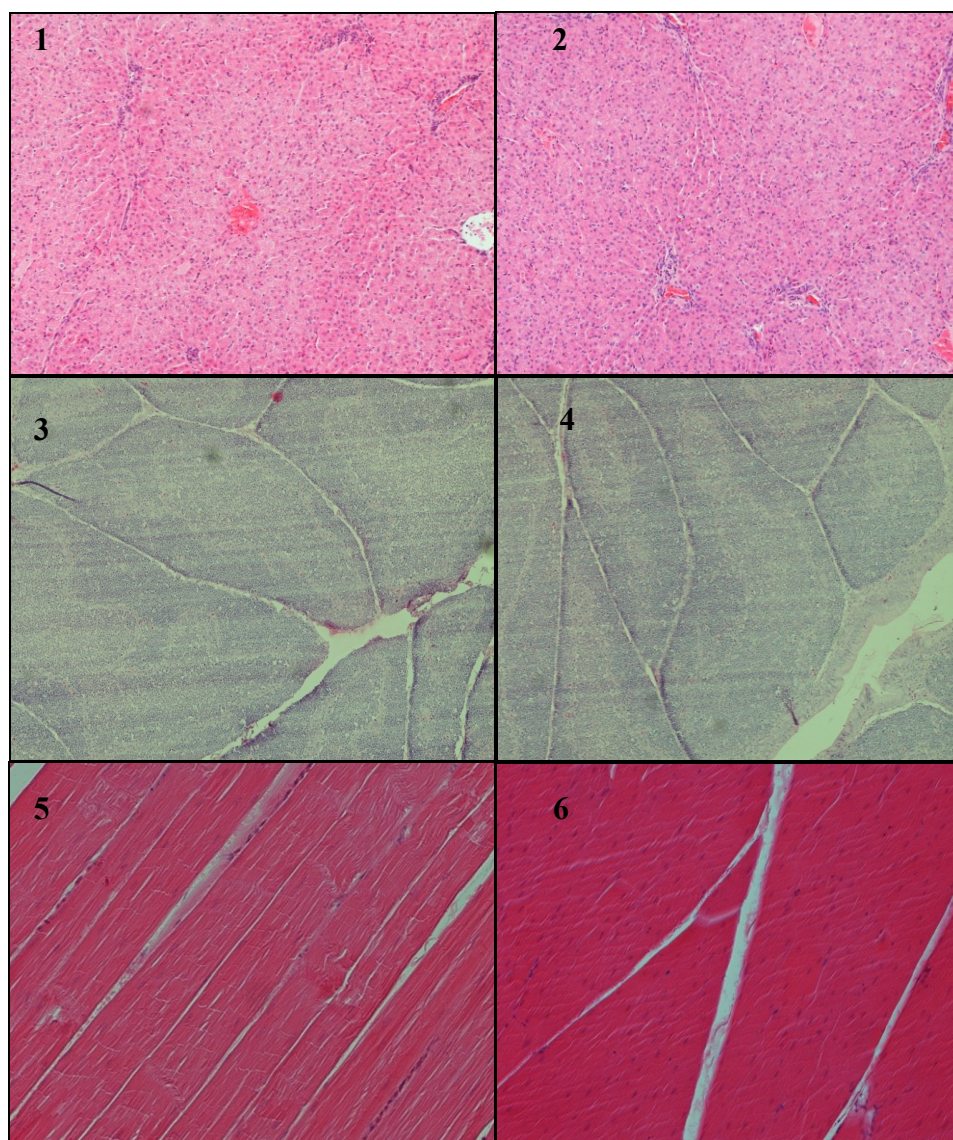
Results

Results of histopathological examination of internal organs of control animals and animals fed GM feed materials are shown in Table 1 and Figs 1-6.

Broiler chickens. Macroscopical and histopathological examinations showed no difference between the control group (diet without GM feed

materials) and the experimental groups (diets with GM feed materials). Histopathological changes were observed in all samples of the liver and kidneys and only in some preparations of the spleen (Table 1). Other organs did not show any histopathological lesions (Table 1, Figs 3-4). The changes were found in a similar range in both the control and experimental groups, regardless of participation in the diet of GM feed materials.

Laying hens. Macroscopical and histopathological examinations of the liver, kidney, spleen, pancreas, duodenum, jejunum, and skeletal muscle showed no significant differences between the control group and experimental groups (Figs 5-6). In case of the liver, kidneys, spleen, pancreas, and skeletal muscles, observed changes were similar in regard to their number and appearance, in both the control group, as well as, experimental groups, irrespective of the tested GM feed components (Table 1).



Figs 1-6. Histopathological examination of tissue sections from experimental animals, HE. 100x. 1. Calf liver, group I – control (focal lymphoid cell infiltrations). 2. Calf liver, group IV – GM fed (focal lymphoid cell infiltrations). 3. Bursa of Fabricius, broilers, group I – control. 4. Bursa of Fabricius, broilers, group IV - GM fed (lack of any lesions). 5. Skeletal muscle, laying hens, group I – control. 6. Skeletal muscle, laying hens, group IV GM fed (lack of any lesions).

Table 1
Results of histopathological examination of internal organs and muscles of control animals
and animals fed genetically modified corn and soybean meal

Animal species	Histopathological changes		Number of animals with histopathological changes/number of animals examined					
			I Control	II GM soybean meal	III GM corn	IV GM soybean meal + GM corn	V Control + cereals	VI GM soybean meal + cereals
Broiler chickens	Liver	ab	10/10	10/10	10/10	10/10		
	Kidney	a	8/10	8/10	7/10	8/10		
		c	10/10	10/10	10/10	10/10		
		d	7/10	7/10	9/10	9/10	n/a	n/a
	Spleen	c	4/10	4/10	5/10	4/10		
		e	4/10	3/10	3/10	3/10		
	Pancreas		0/10	0/10	0/10	0/10		
	Duodenum		0/10	0/10	0/10	0/10		
	Jejunum		0/10	0/10	0/10	0/10		
	Bursa of Fabricius		0/10	0/10	0/10	0/10		
Laying hens	Liver	a	4/10	4/10	3/10	4/10		
		b	4/10	5/10	5/10	5/10		
		c	5/10	3/10	4/10	4/10		
	Kidney	a	7/10	7/10	6/10	7/10		
		c	10/10	5/10	10/10	10/10		
		d	3/10	4/10	6/10	3/10	n/a	n/a
		f	4/10	4/10	7/10	4/10		
	Spleen	a	1/10	2/10	2/10	2/10		
		c	5/10	5/10	3/10	4/10		
	Pancreas	a	2/10	2/10	2/10	2/10		
	Duodenum		0/10	0/10	0/10	0/10		
	Jejunum	g	0/10	0/10	1/10	0/10		
	Muscles	a	5/10	3/10	3/10	5/10		
Calves	Liver	a	1/5	1/5	1/5	1/5		
		b	1/5	2/5	1/5	0/5		
		c	3/5	4/5	4/5	4/5		
	Kidney	a	1/5	2/5	1/5	1/5		
	Spleen		0/5	0/5	0/5	0/5	n/a	n/a
	Pancreas	a	0/5	1/5	0/5	0/5		
	Duodenum		0/5	0/5	0/5	0/5		
	Jejunum		0/5	0/5	0/5	0/5		
	Muscles		0/5	0/5	0/5	0/5		
Pigs	Liver	a	2/6	1/6	2/6	1/6	1/6	1/6
		c	3/6	3/6	6/6	3/6	3/6	3/6
		h	2/6	0/6	0/6	1/6	1/6	2/6

Kidney	a	3/6	3/6	3/6	3/6	3/6	3/6
	c	3/6	3/6	3/6	3/6	3/6	3/6
Spleen	c	3/6	3/6	3/6	3/6	3/6	3/6
Pancreas	a	5/6	5/6	3/6	4/6	4/6	5/6
Duodenum	d	3/6	3/6	3/6	3/6	3/6	3/6
Jejunum	a	3/6	3/6	3/6	3/6	3/6	3/6
	c	3/6	3/6	3/6	3/6	3/6	3/6
	i	3/6	3/6	3/6	3/6	3/6	3/6
Muscles	a	2/6	2/6	0/6	1/6	2/6	0/6

a - lymphoid cell infiltrates

b - foamy structure of a slight degree of hepatocytes

c - congestion of the parenchyma from a slight to moderate degree.

d - local necrosis of epithelial cells

e - increased number of germinal centres

f - presence of localised concentrations of single droplets of fat

g - thinning of the mucosa

h - diffuse infiltration of plasma cells in the moderate degree

i - increased number of eosinophilic granulocytes in the mucosa

n/a - not analysed

Pigs. In some cases, histopathological changes were observed in the liver, kidneys, spleen, pancreas, duodenum, jejunum, and muscles (Table 1).

Calves. Histopathological examination of the liver, kidneys, spleen, pancreas, duodenum, jejunum, and skeletal muscle showed no significant differences between the control group and experimental groups. Occasionally, regardless of the use of GM feed, changes in the liver and kidneys were observed (Table 1, Figs 1 - 2). These changes were found in a similar range in all groups, regardless of the presence of GM components in the diet.

Statistical analysis. Kruskal-Wallis test showed in no uncertain terms the lack of any statistically significant differences between the groups of experimental animals. The value of "P" for multiple comparisons (bilateral) was always set to 1. This indicates that the level of convergence of the results between different experimental groups and the control group is the nearest equal to 1 (on a scale of 0 to 1), meaning that the difference is not significant, the lack of difference is presented in the high probability test ($p=0.6242$ to 0.9998 , is sufficiently distant from the boundary $P=0.05$). Dunnett's test, which is a fairly rigorous test interpreting quantitative values, also did not identify a single significant statistical difference between the results of the control group and the results of other groups - the level of "similarity" (the probability that the results originate from the same sample) ranged from 0.794634 to 1.0 for two-sided test, and from 0.431441 to 0.965332 for one-sided tests. Spearman rank correlation also confirmed the results of these two tests - the correlation coefficient between the control group and the other groups of animals remained at a very high level and varied in the range from 0.733799 to 0.979747 for the data unit 0-1 and 0.720904 , until the 1.0 for the data presenting rate of structure inside of the organ.

Discussion

Histopathological examination is a valid laboratory technique in cases where other diagnostic methods fail. On the other hand, this technique is indispensable in pathomorphological evaluation of side effects of vaccines, drugs, and chemical compounds. The aim of the experiment was to study in a more detailed way the eventual harmful effects of the protein products of the transgene Bt MON810 maize grain and soybean EPSPS enzyme protein MON 40-3-2 (RR) present in the animal feed. Histopathological lesions of the internal organs are usually associated with the disease; however, it is known that fairly often histological changes or "deviations from the physiology" without any documented negative impact on animal health can be found in clinically healthy animals (1, 11, 12). The example might be vacuolar changes in the red nucleus or some other nuclei of the cattle brain of the otherwise BSE negative cattle. Artifacts are another category usually associated with histological processing of tissues that have no relation to the physiological state of the body *e.g.* incidentally observed spongy structure of some hepatocytes as a result of washing out fat deposits.

The study revealed morphological changes in many organs, and therefore statistical analyses aiming to shed light on the eventual link between GM forage feeding and observed lesions were performed. The analysis based on the level of test probability showed no statistically significant differences. The same was confirmed by the analysis performed using ANOVA rank Kruskal-Wallis and median tests. The results confirm the findings of other authors on the lack of harmful effects of GM products in feed materials on animal health. Such conclusion predominates in the majority of publications (8, 10). The report of EFSA experts (4), in which the panel of leading specialists discussed various elements of the safety and nutritional

assessment procedure for genetically modified plant derived food and feed, deserves a great attention, as the conclusions of this report were similar to our observations and other cited studies.

Histopathological changes observed in animals from all experimental groups are likely to be the result of intensive feeding, including a high content of protein and energy metabolism in the diets, and a high metabolic rate especially in fast growing broiler chickens pigs and calves. Frequently observed foamy structure of hepatocytes was likely an artifact specific to the liver and associated with leached glycogen, which was hydrolysed during histological processing of the tissue sections.

It must be concluded that in broilers, laying hens, pigs, and calves fed diets containing tested GM feed materials, these materials had no adverse effect on morphology and structure of internal organs and muscles, as assessed under light microscope. This conclusion was made on the basis of the result of experiment described above in details. The authors did not have the opportunity to conduct prolonged and more advanced research. In particular, carrying out multigeneration study or including other more advanced methodologies e.g. transmission electron microscopy (TEM) or proteomics could be helpful in disclosing more subtle morphological changes in cell structure or proteome alterations (4).

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